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Water Quality Criteria for Colored Smokes:

Solvent Green 3

FINAL REPORT

Kowetha A. Davidson
Patricia S. Hovatter

December 1987

OPERATED BY
MARTIN MARIETTA ENERGY SYSTEMS, INC
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The available data on the environmental fate, aquatic toxicity, and mammalian toxicity of Solvent Green 3, an anthraquinone dye used in colored smoke grenades, were reviewed. The U.S. Environmental Protection Agency (USEPA) guidelines were used in an attempt to generate water quality criteria for the protection of human health and of aquatic life and its uses.

Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. During typical production of pyrotechnic items, approximately 1 to 2 percent of the smoke formulation is released into the aquatic environment. The primary aquatic system receiving these

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discharges is the Arkansas River and associated drainages. The hydrophobic nature of Solvent Green 3 indicates that, when released into aquatic systems, the dye should occur in a particulate form that may be deposited in the bottom sediments.

Sufficient data to determine the toxicity of Solvent Green 3 in freshwater aquatic organisms are lacking. The 96-hr TL_{50} for _Pimephales promelas_ is >100 mg/L. Solvent Green 3, at a concentration of 10 mg/L, causes a transient reduction in growth of the green alga _Selenastrum capricornutum_. No data are available concerning the chronic toxicity or bioaccumulation of Solvent Green 3 in aquatic organisms. However, based on the calculated octanol-water partition coefficient, the dye would be expected to bioaccumulate significantly. According to USEPA guidelines, a Criterion Maximum Concentration and a Criterion Continuous Concentration cannot be determined for this dye. Toxicity tests are recommended using measured concentrations prepared as serial dilutions of a stock solution in which Solvent Green 3 is dissolved in an appropriate solvent. Toxicity studies with burrowing mayflies are also recommended.

No data are available on any of the toxicity parameters for Solvent Green 3 in humans.

No data on the pharmacokinetics of Solvent Green 3 administered orally in laboratory animals are available. Solvent Green 3 administered by inhalation is retained in the respiratory tract, with half-times of clearance after a 90-day exposure of 277 days in male rats and 288 days in female rats.

Solvent Green 3 has a low order of toxicity whether administered by the oral, dermal, or inhalation route. The acute oral LD_{50} is >3.16 g/kg in rats, but may be as high as 15 g/kg, >1 g/kg in dogs, and 10 g/kg in rabbits. Solvent Green 3 is not irritating to eyes and is only mildly irritating to the skin. Subchronic and chronic inhalation exposure to Solvent Green 3 in mixtures with other dyes causes effects indicative of respiratory tract inflammation.

Solvent Green 3 alone is not mutagenic in the Salmonella Reversion Assay. The dye, tested in a mixture with Solvent Yellow 33, gives positive mutagenic responses in the Salmonella Reversion Assay and the Mouse Lymphoma Assay; the data indicate that the positive responses may be attributed to Solvent Yellow 33. Solvent Green 3 is not teratogenic in rats or rabbits, and the dye is also not carcinogenic in the Mouse Lung Tumor Bioassay.

Sufficient pertinent data were not available for deriving a water quality criterion for the protection of human health.

WATER QUALITY CRITERIA FOR COLORED SMOKES:
SOLVENT GREEN 3

FINAL REPORT

Kowetha A. Davidson
Patricia S. Hovatter

Chemical Effects Information Task Group
Information Research and Analysis Section
Health and Safety Research Division

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EXECUTIVE SUMMARY

Solvent Green 3 is an anthraquinone dye used by the military in M18 green smoke grenades that are deployed for communication. Solvent Green 3, certified and approved for use in contact lenses and externally applied drugs and cosmetics, is known as D & C Green No. 6. The dye is industrially prepared by reacting toluidine with 1,4-dihydroxy-2,3-dihydroanthraquinone, followed by oxidation.

The environmental release of Solvent Green 3 may occur during manufacturing, during formulation and loading of smoke grenades, or upon detonation of grenades during training and testing operations. Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. The primary aquatic systems receiving wastewaters at the arsenal are the Arkansas River and associated drainages. Prior to the installation of a pollution abatement facility in 1979, contamination to this system from untreated pyrotechnic wastes was reported as significant.

Limited information is available concerning the environmental fate of Solvent Green. The hydrophobic nature of the dye indicates that when released into aquatic systems it should occur in a particulate form that may be deposited in the bottom sediments.

Sufficient data to determine the toxicity of Solvent Green 3 in freshwater aquatic organisms are lacking. The 96-hr TL_{50} for _Pimephales promelas_ is >100 mg/L, which would indicate that the dye is relatively nontoxic. Static acute tests with fish and invertebrates exposed to a Solvent Yellow 33/Solvent Green 3 mixture (30:70), provide limited and inconsistent information. Mortality was observed in only one (rainbow trout) of the eight species tested at solubility limits, 0.076 mg/L Solvent Yellow 33 and <0.002 mg/L Solvent Green 3. These data are not acceptable for determining the toxicity of Solvent Green 3 in fish and aquatic invertebrates because: (1) data for a mixture cannot be used to determine the toxicity of a single compound and (2) Solvent Green 3 is insoluble in water and was not detected in test solutions by high performance liquid chromatographic (HPLC) analysis at a detection limit of 0.002 mg/L. Because sufficient data are not available, U.S. Environmental Protection Agency (USEPA) guidelines could not be used to calculate a Final Acute Value for the dye, and, consequently, a Criterion Maximum Concentration could not be determined. Due to the possible deposition of the dye in aquatic sediments, toxicity studies with burrowing mayflies are recommended.

Solvent Green 3, at a concentration of 10 mg/L, causes a transient reduction in growth of the green alga _Selenastrum capricornutum_. No toxic effects were observed at 1 mg/L. The Solvent Yellow 33/Solvent Green 3 mixture significantly reduces algal growth at the estimated aqueous solubility limits, 0.198 mg/L Solvent Yellow 33 and <0.002 mg/L Solvent Green 3. As explained above, these data are also not acceptable for determining the toxicity of Solvent Green 3 in aquatic plants. Additional testing of _Selenastrum capricornutum_, using known measured concentrations

of Solvent Green 3 alone is required in order to calculate a Final Plant Value according to USEPA guidelines.

No data are available on the chronic toxicity or bioaccumulation of Solvent Green 3 in aquatic organisms; therefore, Final Chronic and Final Residue Values cannot be determined and a Criterion Continuous Concentration cannot be established.

No data are available on any of the toxicity parameters for Solvent Green 3 in humans.

No data are available on the pharmacokinetics of Solvent Green 3 administered orally in laboratory animals, but Solvent Green 3 administered by inhalation is retained in the respiratory tract. The half-time of clearance after a 90-day exposure is 277 days in male rats and 288 days in female rats.

Solvent Green 3 exhibits a low order of toxicity whether administered by the oral, dermal, or inhalation route. The acute oral LD_{50} is >3.16 g/kg, but may be as high as 15 g/kg in rats, >1 g/kg in dogs, and 10 g/kg in rabbits. Solvent Green 3 is not irritating to eyes and is only mildly irritating to the skin. The acute LCt_{50} for five experimental animal species combined (monkeys, dogs, swine, rabbits, and rats) is 319,447 mg·min/m^3. Subchronic and chronic inhalation exposure to Solvent Green 3 in mixtures with other dyes causes effects indicative of respiratory tract inflammation.

Solvent Green 3 alone is not mutagenic in the Salmonella Reversion Assay. When the dye is tested in a mixture with Solvent Yellow 33, positive mutagenic responses are observed in the Salmonella Reversion Assay and the Mouse Lymphoma Assay, but the data indicate that Solvent Yellow 33 may be responsible for the positive responses. Solvent Green 3 is neither teratogenic in rats or rabbits, nor carcinogenic in the Mouse Lung Tumor Bioassay.

Pertinent data on carcinogenicity of Solvent Green 3 in laboratory animals or humans were not available. Data on chronic toxicity were also unavailable. Therefore, USEPA guidelines could not be used to derive a water quality criterion for the protection o human health.

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ACKNOWLEDGMENTS

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1. INTRODUCTION

Solvent Green 3 is an anthraquinone dye used by the military in M18 green smoke grenades that are deployed for communication. Solvent Green 3, certified and approved by the U.S. Food and Drug Administration (USFDA) for use in contact lenses and externally applied drugs and cosmetics, is known as D & C Green No. 6 (USFDA 1984). D & C Green No. 6 is also approved for the coloring of polyethylene terephthalate and polyglycolic acid surgical sutures that are used in general and ophthalmic surgery (USFDA 1984). The primary commercial uses of the dye are for the coloring of textiles (Dacre et al. 1979) and plastics (Webber 1979). The name Solvent Green 3 will be used throughout this document to refer to both the dye used by the military and that certified and approved by the USFDA.

The pyrotechnic composition of colored smoke grenades consists of the dye mixture, oxidizer, fuel, coolant, and diatomaceous earth as a binder. Each grenade contains approximately 352 g of the dye mixture, which is formulated at the Aberdeen Proving Ground, Maryland (Smith and Stewart 1982). The cooling agent is used to prevent excessive decomposition of the organic dye due to heat produced by the fuel. Upon detonation of the grenade, heat from the burning fuel causes the dye to volatilize, which then condenses outside the pyrotechnic, thereby producing smoke. The burning time is adjusted by the proportion of fuel and oxidizer and by the use of the cooling agent (Cichowicz and Wentsel 1983).

The production and use of green smoke grenades could result in environmental contamination and human exposure to Solvent Green 3 and its combustion products. Therefore, the objectives of this report are to review the available literature on the environmental fate, aquatic toxicity, and mammalian toxicity of Solvent Green 3 and to generate water quality criteria for the protection of human health and of aquatic life and its uses. Current U.S. Environmental Protection Agency (USEPA) guidelines used to derive these criteria are summarized in the appendixes.

1.1 PHYSICAL AND CHEMICAL PROPERTIES

Solvent Green 3 is insoluble in water. Fisher et al. (1985) used high performance liquid chromatographic (HPLC) methods to determine the solubility of technical grade Solvent Green 3 in diluent freshwater with a mean pH of 7.6, alkalinity of 156 mg/L as $CaCO_3$, and hardness of 180 mg/L as $CaCO_3$. Solubility was determined at the three temperatures to be used in toxicity tests--12, 17, and 22°C. Solvent Green 3 was not detected at any test temperature, indicating a solubility of <0.08 mg/L, the detection limit of the HPLC system.

Other physical and chemical properties of Solvent Green 3 are as follows:

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CAS registry No.:	128-80-3
Color index (CI) No.:	61565 (Colour Index 1971)
Chemical name:	Solvent Green 3
Synonyms, trade names:	1,4-bis-[(4-methylphenyl)amino]-9,10-anthracenedione (9 C.I.), 1,4-di-p-toluidino-anthraquinone (8 C.I.), D and C Green No. 6, Quinizarin Green SS, Alizarine Green G Base, Arlosol Green B, Nitrofast Green GB, Waxoline Green G, Organol Green J, Sudan Green 4B (MEDLARS[RTECS] 1986)

Structural formula:

Molecular formula:	$C_{28}H_{22}N_2O_2$
Molecular weight:	418.50 (Windholz 1983)
Physical state:	Solid at 20°C (Cichowicz and Wentsel 1983); Dark violet needles (Windholz 1983)
Melting point:	218°C (Windholz 1983); 220-221°C (Aldrich 1984); 490°K (Kharitonova et al. 1984)
Initial decomposition temperature (°K):	573 (Kharitonova et al. 1984)
Apparent density (g/mL):	(Spec.) 0.45 ± 0.25 (Cichowicz and Wentsel 1983)
Solubility in other solvents	Soluble in 3.4 g/100mL alcohol at 25°C, and in hydrochloric and sulfuric acid (Dacre et al. 1979); soluble in benzene, toluene, and butyl acetate; moderately soluble in mineral wax, petroleum jelly, stearic acid, oleic acid, and mineral oil; sparingly soluble in acetone, methanol, ethanol, ethyl ether (Zuckerman and Senackerib 1979)
Octanol-water partition coefficient ($\log K_p$):	6.657 (G.L. Baughman, USEPA, personal communication 1987)

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Absorption (λmax in nm):   644 (Aldrich 1984)

Heat of reaction (cal/g):   299 for green smoke mixture (Scanes and Martin 1974)

Activation energy for
thermal decomposition
(kJ/mol):   183.7 (Kharitonova et al. 1984)

Relative permittivity (ϵ_r):   3.19 (Lewin and Chapman 1977)

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

1.2.1 Manufacturing and Production

Solvent Green 3 is industrially prepared by reacting toluidine with 1,4-dihydroxy-2,3-dihydroanthraquinone, followed by oxidation (Rys and Zollinger, 1970 as reported in Kitchens et al. 1978). The dye is produced in the United States by Atlantic Chemical Industries in Nutley, New Jersey, and Toms River Chemical in Toms River, New Jersey (S.R.I. 1977).

U.S. production in 1973 was 217,000 lb (U.S.I.T.C. 1975, as reported in Dacre et al. 1979), and importation was 4,760 lb (U.S.T.C. 1974, as reported in Dacre et al. 1979). U.S. production of Solvent Green 3 in 1982 was >15,179 lb (U.S.I.T.C. 1983a, as reported in Sigman et al. 1985), and importation was 13,616 lb (U.S.I.T.C. 1983b, as reported in Sigman et al. 1985). The USFDA certified an average of 1,790 lb of D & C Green No. 6 per year during the period between 1971 and 1980, with declining use in the second half of the decade (USFDA 1982).

Estimated civilian production capacity for Solvent Green 3 is 500,000 to 750,000 lb/yr (Kitchens et al. 1978). The average annual use of Solvent Green 3 by the U.S. Army at the Pine Bluff Arsenal during the period between 1965 and 1975 was 55,400 lb/yr (Burrows 1977, as reported in Kitchens et al. 1978). The 1978 production schedule of green smoke grenades at Pine Bluff Arsenal required 64,000 lb or about 5,300 lb/month. The full mobilization rate would approximately double this rate (Kitchens et al. 1978). Consequently, the Army would use 8.5 to 13 percent of the estimated civilian production capacity during normal operations and 17 to 26 percent at full mobilization (Kitchens et al. 1978).

Green smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas, using the Glatt Mixing Process. A fluidized bed granulator combines the three operations of mixing, granulation, and drying. This technique reduces cost, improves efficiency, and provides better engineering controls for material containment, thereby reducing worker exposure to dust and the pollutant discharge of acetone (Garcia et al. 1982). The formulation of the green smoke grenade is as follows: 29.5 percent Solvent Green 3, 12.5 percent Solvent Yellow 33, 24.5 percent potassium chlorate (oxidizer), 17.0

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percent magnesium carbonate (coolant), and 16.5 percent powdered sugar (fuel) (Smith and Stewart 1982).

1.2.2 Analytical Methods

Major and minor components of colored smoke mixtures can be separated and identified by various methods dependent upon the solubility and volatility of the major components. These techniques include thin-layer chromatography (TLC), HPLC, combined gas chromatography/mass spectrometry, nuclear magnetic resonance spectroscopy, and fluorescence spectrometry (Rubin and Buchanan 1983).

Several investigators have analyzed Solvent Green 3, using TLC and visible spectroscopy. Absorbents that have been used include cellulose, silica gel, magnesia, and florisil (Prandi 1970, as reported in Dacre et al. 1979). Penner (1968) used the following solvent systems for analyzing coal tar color additives, including Solvent Green 3: (1) ethyl acetate-n-butanol-pyridine-water (5:5:6:5), (2) ethyl acetate-n-butanol-concentrated ammonia (20:55:25), (3) ethyl acetate-n-propanol-concentrated ammonia-water (35:35:20:20), and (4) n-propanol-ethyl acetate-concentrated ammonia (65:75:60). Rai (1971) performed TLC analysis on fat-soluble dyes using the following eluents: benzene, xylene, petroleum ether-acetone (40:60), and petroleum ether-chloroform (40:60).

Fadil and McSharry (1979) extracted and separated Solvent Green 3 from tablet-coating formulations. The formulation was treated with phosphoric acid, dissolved in methanol, and made alkaline with ammonium hydroxide. The solution was then centrifuged, and the supernate was analyzed by TLC on silica gel plates, using ethyl acetate-methanol-water-concentrated ammonium hydroxide (150:40:35:5) as a solvent.

Large-scale separations of Solvent Green 3 have been performed using column chromatography (Jones 1966, as reported in Dacre et al. 1979).

The results of TLC and ultraviolet spectroscopic analyses of two samples of Solvent Green 3 obtained from Edgewood Arsenal showed that the samples were 92 percent pure (Blumbergs et al. 1973). Purity was raised to 100 percent by recrystallization twice with toluene.

Fisher et al. (1985) used reverse-phase HPLC (C_{18} column) with an isocratic 10 percent distilled water: 90 percent methanol mobile phase to measure concentrations of the individual dyes in a Solvent Yellow 33/Solvent Green 3 mixture. The detection limit by HPLC analysis was 0.08 mg/L. The retention time for the green component ranged from 20.69 to 21.0 min. Three minor contaminants were associated with Solvent Green 3; however, these were not identified. An HPLC analysis performed by the same method by Henderson et al. (1984a) identified the impurities associated with the Solvent Green 3 component of the dye mixture as quinazarin and p-toluidine.

Rubin and Buchanan (1985) demonstrated that following separation, ^{13}C nuclear magnetic resonance could be used to identify Solvent Green 3 and

other fractions of mixtures of anthraquinone-derived dyes. Deuterated chloroform at concentrations of 30 mg/ml was used as the solvent.

Bertocchi et al. (1980) used flameless atomic absorption spectrometry to determine that 1.2-g samples of Solvent Green 3 contained 0.21 ppm of mercury.

Chin and Borer (1983) studied the combustion products from colored smokes containing organic dyes. The organic vapors and particulates in the effluent were collected using a modified EPA-Method 5 sampling train containing Amberlite XAD-2 as the absorbent. Analytical methods used to separate, purify, and identify the products included TLC, HPLC, gas chromatography, combined gas chromatography/mass spectrometry, and nuclear magnetic resonance.

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

No information was found in the literature concerning the abiotic effects of Solvent Green 3.

2.2 ENVIRONMENTAL FATE

2.2.1 Sources and Transport

Solvent Green 3 may be released into the environment during manufacture of the dye, during formulation and loading of the colored smoke grenade, or during training and testing operations. Kitchens et al. (1978) reported that during typical production of pyrotechnic items, approximately 1 to 2 percent of the smoke formulation or an estimated 53 to 106 lb of Solvent Green 3 would be discharged into receiving waters within the area of Pine Bluff Arsenal. At full mobilization, 107 to 214 lb/month would be released (Kitchens et al. 1978). Combustion products resulting from detonation of the grenades can enter the aquatic environment as fallout, through runoff, or by leaching from soils (Cichowicz and Wentsel 1983).

Four main aquatic systems that could receive pyrotechnic discharges drain into the Arkansas River, which fronts the Arsenal for approximately six miles. Three of the aquatic systems originate on the installation. They are: Triplett Creek, Yellow Creek with associated drainages, and McGregor Reach. The fourth, Eastwood Bayou, originates off the installation. An aquifer also occurs below the Arsenal. The pyrotechnic complex is located just southwest of Yellow Lake. Prior to the installation of a pollution abatement facility in 1979, untreated pyrotechnic wastes were discharged directly into these receiving aquatic systems, indicating that past contamination was significant (Kitchens et al. 1978). Pinkham et al. (1977, as reported in Kitchens et al. 1978) reported contamination, including pyrotechnic residues and smoke mixtures, within Yellow Lake and within a munitions test area on the Arkansas River. Fortner et al. (1979, as reported in Kitchens et al. 1978) reported, however, that the wastewater treatment facility will reduce effluent discharges to acceptable levels.

Solvent Green 3 is insoluble in water and therefore, should occur primaril in a particulate form in aquatic systems either suspended in the water column or deposited in the bottom sediment.

2.2.2 Degradation and Transformation

Limited information was available concerning the physical, chemical, or biological degradation and/or transformation of Solvent Green 3. Cichowicz and Wentsel (1983) reported that the dye will undergo photodecomposition at rates dependent on the surrounding environmental conditions. Deiner (1982)

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stated that colored smokes, disseminated by grenades, were degraded by oxidation.

Investigations by Chin and Borer (1983) and Chin et al. (1984) indicate that during combustion, 90 to 95 percent of the organic dye in green-, yellow-, orange-, and red-colored smokes sublimes, while 5 to 10 percent decomposes to polynuclear organic materials, including polynuclear aromatic hydrocarbons. The decomposition products are the result of uncombusted impurities and the thermal decomposition and thermal rearrangement of the parent dye. The investigators observed no ring openings during decomposition. Chin and Borer (1983) showed that during combustion, 5 to 10 percent of Solvent Green 3 decomposes to smaller anthraquinone derivatives through step-wise thermal degradation as illustrated in Figure 1. TLC analysis identified some of the effluents from combustion of Solvent Green 3 as 1-p-toluidinoanthraquinone, 1-p-toluidino-4-aminoanthraquinone, p-toluidine, 1-aminoanthraquinone, and 1-p-toluidinoanthrone (Chin and Borer 1983, Chin et al. 1984).

2.3 SUMMARY

The production and use of Solvent Green 3 may result in the release of the dye and its combustion products to the environment. The primary aquatic systems receiving wastewaters from the production of green smoke grenades at the Pine Bluff Arsenal, Arkansas, are the Arkansas River and associated drainages. Past contamination of pyrotechnic residues to these systems was reported as significant; however, wastewater treatment begun in 1979 should reduce effluent discharges to acceptable levels. Limited information is available concerning the environmental fate of Solvent Green 3; however, the hydrophobic nature of the dye indicates that it should occur in a particulate form when released to aquatic systems.

Solvent Green 3 photodecomposes at rates dependent on the surrounding environmental conditions. During combustion of an organic dye, 90 to 95 percent sublimes, while 5 to 10 percent of the dye undergoes thermal decomposition and rearrangement, but, no ring openings occur. Major decomposition products include polynuclear aromatic hydrocarbons and polynuclear organic materials.

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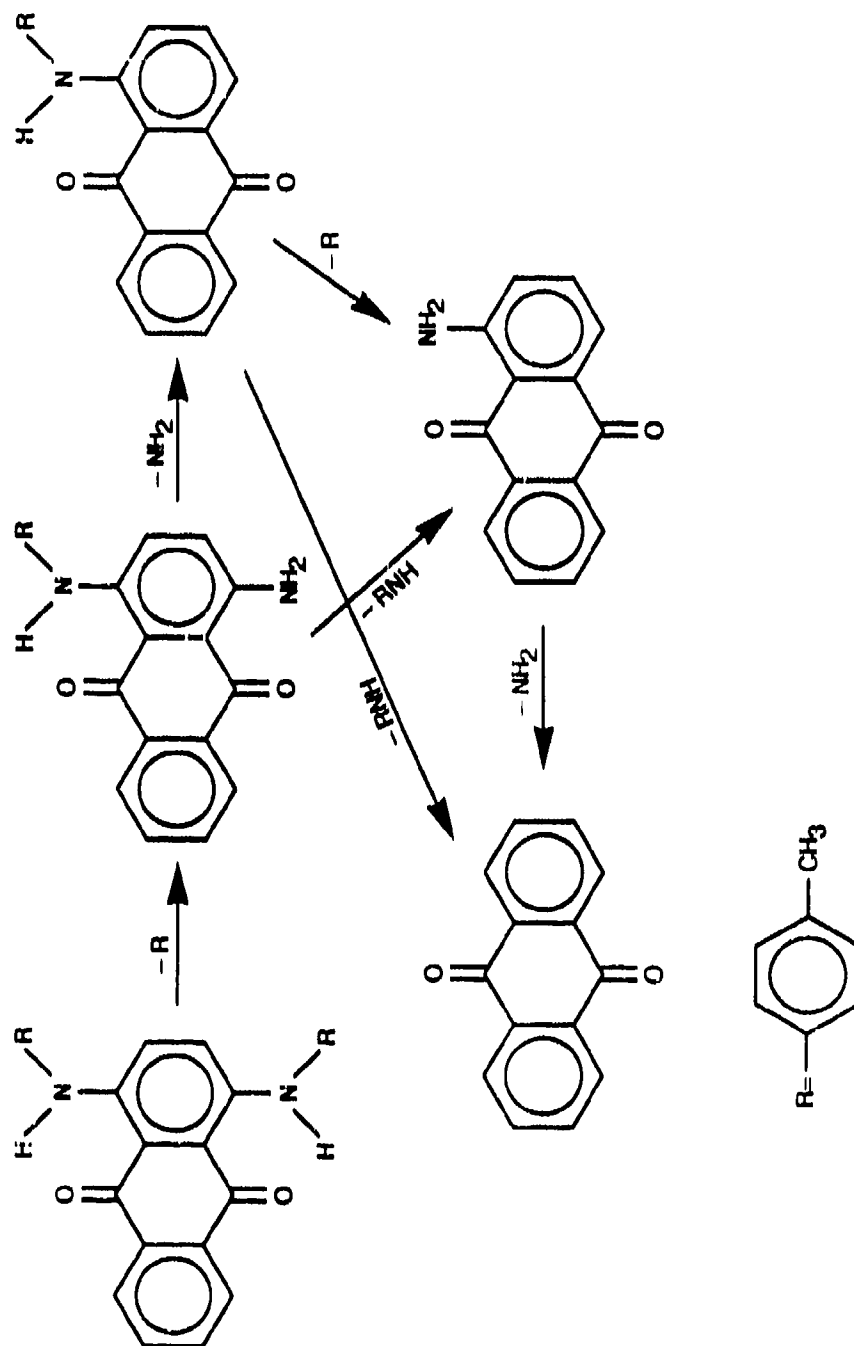
Figure 1. Thermal Degradation Pattern of Solvent Green 3 Between 400 and 600°C. (From Chin and Borer 1983).

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3.1 ACUTE TOXICITY IN ANIMALS

Chillingworth (1974) conducted static acute bioassays to determine the mean tolerance limit (TL_{50}) of *Pimephales promelas* (fathead minnow) to Solvent Green 3. Tests were conducted following accepted standard procedures. Test concentrations were determined from preliminary range-finding tests. Due to its insolubility, the dye was dispersed with the solvent Roax 83-A to form a 15-percent-by-weight dispersion. Ten fish per group were exposed for 96 hr to the following concentrations: 10, 18, 32, 56, or 100 mg/L. Length of the test fish ranged from 4.3 to 6.1 cm, and their weight ranged from 0.38 to 1.40 g. Measurements of temperature and pH at 0, 48, and 96 hr indicated that these parameters remained relatively constant during testing. Dissolved oxygen (DO) decreased during testing; therefore, the tanks were aerated for 5 min at 48 hr. Alkalinity of the dilution water prior to testing measured 19 mg/L as $CaCO_3$. The results showed that the 96-hr TL_{50} for *Pimephales promelas* was >100 mg/L.

Fisher et al. (1937) studied the acute toxicity of a Solvent Yellow 33/Solvent Green 3 (30:70 ratio) mixture in eight aquatic species of fish and invertebrates. Fish species tested were *Pimephales promelas*, *Ictalurus punctatus* (channel catfish), *Lepomis macrochirus* (bluegill), and *Salmo gairdneri* (rainbow trout). Invertebrate species tested were *Daphnia magna* (water flea), *Gammarus pseudolimnaeus* (amphipod), *Hexagenia bilineata* (mayfly larvae), and *Paratanytarsus parthenogeneticus* (midge larvae). All species were to be tested at the aqueous solubility limit of the dyes at various test temperatures, as determined by HPLC analysis (Fisher et al. 1985). The Solvent Green 3 component of the dye mixture was not detected at either temperature by HPLC analysis at a detection limit of 0.08 mg/L. Using a C-18 Sep-Pak cartridge, the investigators increased the sensitivity of the HPLC system to a detection limit of 0.002 mg/L, but were still unable to detect the Solvent Green 3 component. The authors, therefore, established the solubility limit of the dye as less than the detection limit, either <0.08 mg/L or <0.002 mg/L. Solubility of the Solvent Yellow 33 component of the green smoke mixture ranged from 0.09 ± 0.009 at 12°C to 0.17 ± 0.031 mg/L at 22°C. Dye concentrations were measured at the beginning and end of each test. Static (96 hr for fish; 48 hr for invertebrates) acute bioassays were performed according to American Society for Testing and Materials (ASTM 1980) methods. Preliminary tests with *Daphnia magna* and *Paratanytarsus parthenogeneticus* indicated no toxicity at the established solubility limits for the Solvent Yellow 33/Solvent Green 3 mixture.

Temperature, pH, and total hardness remained relatively constant during testing. In tests with bluegill, DO decreased from 8.5 mg/L at the start of the test to 4.0 mg/L at the end of the test. In tests with rainbow trout, DO decreased from 9.4 to 7.1 mg/L.

Results of the static acute tests with fish and invertebrates are given in Table 1. No mortality was observed in seven of the eight species tested.

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TABLE 1. ACUTE TOXICITY OF SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE
IN EIGHT FRESHWATER AQUATIC SPECIES[a]

Species	Age/Mean Size	Test Temperature (°C)	Test Concentration (mg/L)	Mortality (%)
Daphnia magna	<24 hr	22 ± 2	0.18/<0.08	0
Gammarus pseudolimnaeus	Early young	17 ± 2	0.10/<0.08	0
Hexagenia bilineata	Late instar	22 ± 2	0.17/<0.08	0
Paratanytarsus parthenogeneticus	2nd-3rd instar	22 ± 2	0.18/<0.08	0
Pimephales promelas	Length 10.3 mm Weight 8.0 mg	22 ± 2	0.17/<0.08	0
Ictalurus punctatus	Length 14.2 mm Weight 30.1 mg	22 ± 2	0.11/<0.08	0
Lepomis macrochirus	Length 18.3 mm Weight 108 mg	22 ± 2	0.12/<0.08	0
Salmo gairdneri	Length 23.8 mm Weight 149 mg	12 ± 2	0.076/<0.002	50

a. Adapted from Fisher et al. 1987.

However, in two separate 96-hr static acute tests, 50 percent mortality was observed in rainbow trout exposed to the dye mixture containing <0.002 mg/L of Solvent Green 3 and 0.076 ± 0.004 mg/L of Solvent Yellow 33. No mortality of rainbow trout was observed in tests with 0.089 mg/L of Solvent Yellow 33 alone or with a 50-percent dilution of the dye mixture solution, which contained <0.002 mg/L of Solvent Green 3 and 0.055 mg/L of Solvent Yellow 33. Due to the uncertainty concerning the actual concentration of Solvent Green in the test solution, these results are questionable. The tests should be repeated using known concentrations of purified Solvent Green 3 obtained by dissolving the dye in an appropriate solvent and diluting this stock solution to the desired series of concentrations.

3.2 CHRONIC TOXICITY IN ANIMALS

No information was found in the literature concerning the chronic toxicity of Solvent Green 3 in aquatic organisms.

3.3 TOXICITY IN MICROORGANISMS AND PLANTS

Chillingworth (1974) used the Algal Assay Bottle Procedure (NERP 1971, as reported in Chillingworth 1974) to determine the toxicity of Solvent Green 3 in the green alga Selenastrum capricornutum. Tests were conducted at 24 ± 1°C in triplicate for each concentration; 0, 1, and 10 mg/L. The dye was dispersed with the solvent, Reax 83-A, to form a 15-percent-by-weight dispersion. Each test flask containing 100 ml of nutrient media was inoculated with 1 ml of stock culture containing 10^5 cells in order to obtain an initial concentration of 10^3 cells/ml. Duplicate cell counts were made for each flask at 7 and 14 days. No toxic effects were observed at the 1 mg/L concentration. The 10 mg/L concentration of Solvent Green 3 initially reduced algal growth to 17.8 percent of the control at 7 days. However, recovery was almost complete (92.6 percent of control) by 14 days.

Fisher et al. (1987) studied the effect of the mixture of Solvent Yellow 33/Solvent Green 3 (30:70 ratio) on the growth of the green alga Selenastrum capricornutum. Tests were conducted at 24 ± 1°C with dye concentrations at the established solubility limits, 0.198 mg/L of Solvent Yellow 33 and <0.002 mg/L of Solvent Green 3. As explained in Section 3.1, the Solvent Green 3 component of the mixture was not detected in solution by HPLC analysis with a detection limit of 0.002 mg/L. Sterile assay media was inoculated with cells in log growth (8-day-old stock cultures). Cell density (cells/mL) and biomass (chlorophyll a content expressed in $\mu g/L$) were measured at 0, 24, 48, 72, 96, and 120 hr.

According to Fisher et al. (1987), neither an algistatic or algicidal concentration was observed since cell densities were significantly greater after 5 days than at the time of inoculation. Nevertheless, after the 5-day exposure period, the Solvent Yellow 33/Solvent Green 3 mixture had significantly reduced cell density and biomass by 98 and 99 percent, respectively, of control levels (Table 2, Figures 2 and 3). In a separate test,

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TABLE 2. DENSITY AND BIOMASS OF Selenastrum capricornutum EXPOSED TO
SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE[a]

	Density (cells/mL)					
Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Solvent Yellow 33/ Solvent Green 3 Solubility Limit	5,204	11,984	11,111	12,505	14,896	17,220
Control	5,201	20,274	60,609	224,790	585,190	931,947

	Biomass (µg/L)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Solvent Yellow 33/ Solvent Green 3 Solubility Limit	0.51	1.63	1.41	0.51	0.85	0.51
Control	0.74	5.76	24.35	51.54	98.88	88.76

a. Adapted from Fisher et al. 1987.

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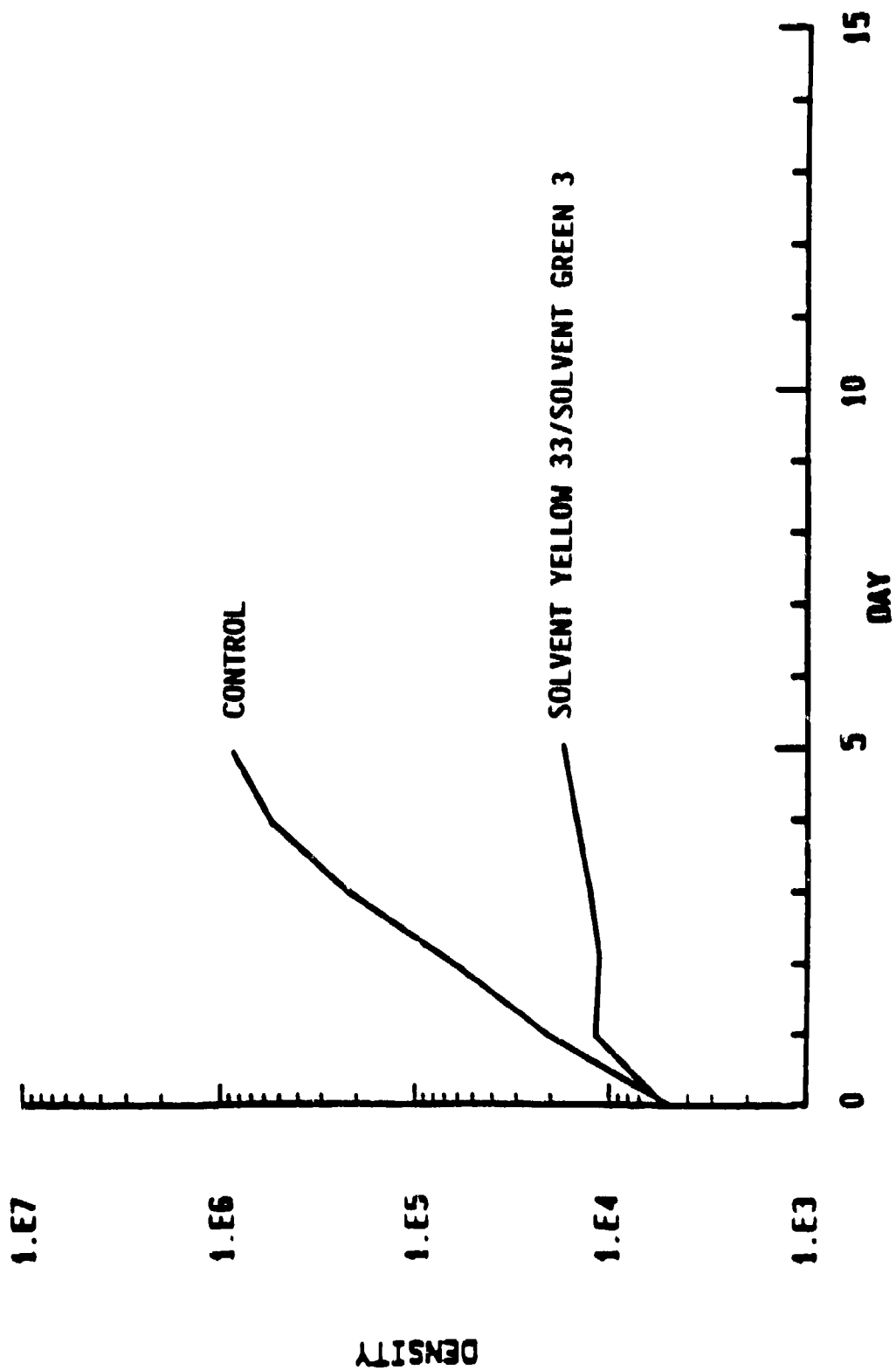
Figure 2. The Effects of Solvent Yellow 33/Solvent Green 3 Mixture on the Growth of _Selenastrum capricornutum_ as Measured by Density (cell/mL). Adapted from Fisher et al. 1987.

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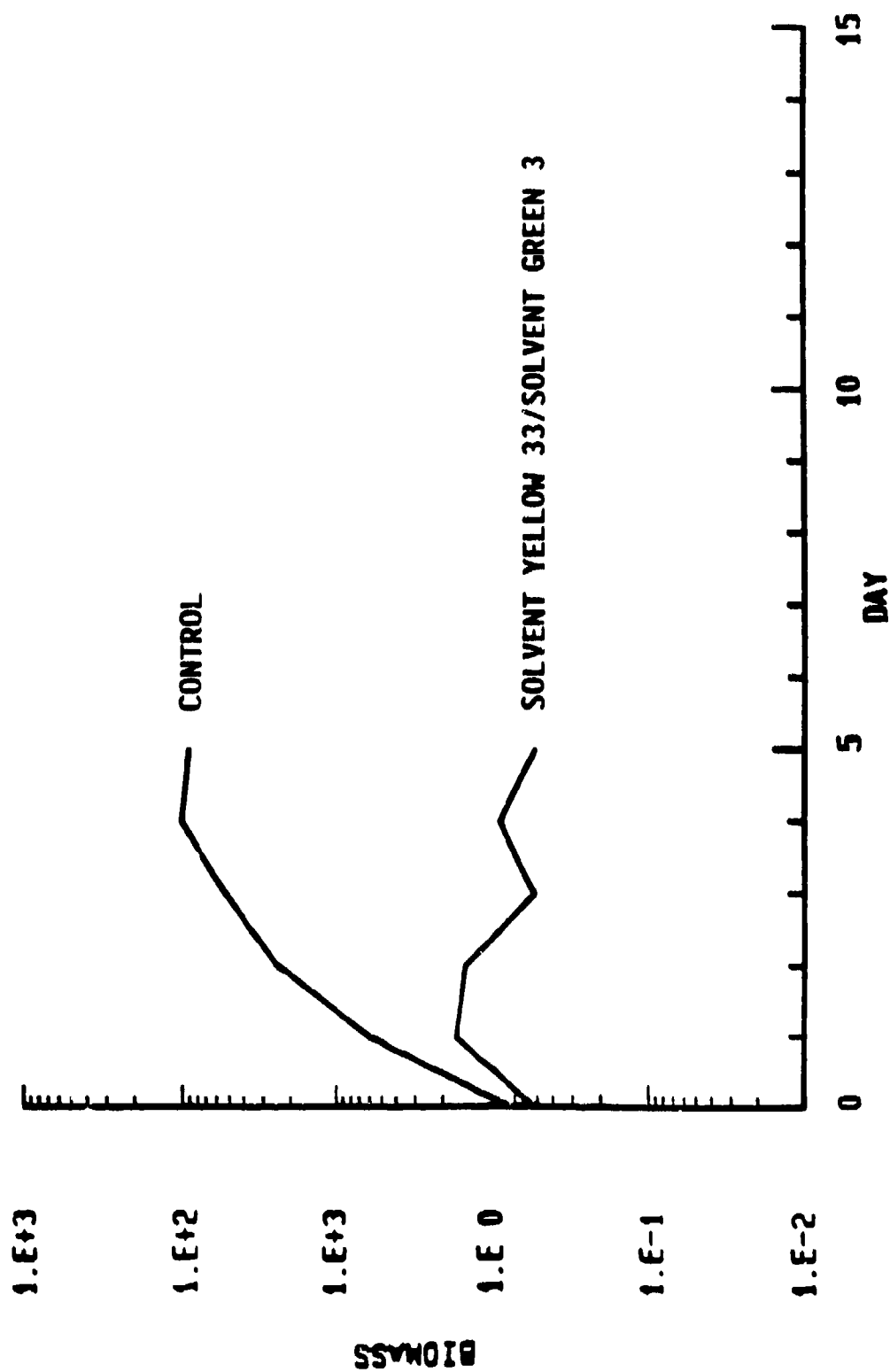
Figure 3. The Effects of Solvent Yellow 33/Solvent Green Mixture on the Growth of _Selenastrum capricornutum_ as Measured by Biomass (μg/L chlorophyll _a_). Adapted from Fisher et al. 1987).

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Solvent Yellow 33 alone at a concentration of 0.20 ± 0.013 mg/L significantly reduced cell density by 68 percent and biomass by 75 percent of control levels. However, due to the uncertainty concerning the actual concentration of Solvent Green 3 in the test solution, these results are questionable. The study should be repeated with known concentrations of purified Solvent Green 3 obtained by dissolving the dye in an appropriate solvent and diluting this stock solution to the desired series of concentrations.

3.4 BIOACCUMULATION

No information was found in the literature concerning the bioaccumulation of Solvent Green 3 by aquatic organisms. However, the calculated octanol-water partition coefficient for the dye is 6.657 (G.L. Baughman, USEPA, personal communication 1987). The value was calculated by the substituent approach of Leo et al. (1971), based on computations used in the computer program CLOGP. Therefore, according to O'Bryan and Ross (1986), Solvent Green 3 would be expected to bioaccumulate significantly, with an estimated bioconcentration factor of ≥ 1000.

3.5 OTHER DATA

Little et al. (1974) investigated the acute toxicity of selected commercial dyes in Pimephales promelas and found that pH may affect the toxicity by influencing the degree of ionization and the site of action of the dye within the organism. Consequently, if the dye is discharged along with acidic or alkaline substances, the toxic effect may be altered.

3.6 SUMMARY

Sufficient data to determine the toxicity of Solvent Green 3 in freshwater aquatic organisms are lacking. The 96-hr TL_{50} for Pimephales promelas is >100 mg/L, which would indicate that the dye is relatively nontoxic. Static acute tests with fish and invertebrates exposed to a Solvent Yellow 33/Solvent Green 3 mixture (30:70) at the established solubility limits provide limited information. No mortality was observed in seven of the eight species tested. However, 50 percent mortality was observed in rainbow trout in two separate 96-hr static acute tests at a concentration of 0.076 mg/L of Solvent Yellow 33 and <0.002 mg/L of Solvent Green 3. No mortality was observed in rainbow trout with a 50 percent dilution of the dye mixture or with Solvent Yellow 33 alone at a concentration of 0.089 mg/L. It is questionable whether this toxicity is due to exposure to Solvent Green 3 because the dye was not detected in solution by HPLC analysis at a detection limit of 0.002 mg/L.

Toxicity tests with Selenastrum capricornutum show that 10 mg/L of Solvent Green 3 initially reduced algal growth to 17.8 percent of the control within 7 days, but recovery was almost complete (92.6 percent of control) by 14 days. The mixture of Solvent Yellow 33/Solvent Green 3 significantly

reduced algal growth at the established aqueous solubility concentrations of
0.198 mg/L of Solvent Yellow 33 and <0.002 mg/L of Solvent Green 3.

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINETICS

4.1.1 Animal Data

Marrs (1983) exposed female Porton mice, female Wistar rats, and female Dunkin-Hartley guinea pigs to a smoke mixture containing 19 percent Solvent Green 3, 13 percent Solvent Yellow 33, and 16 percent Disperse Red 9 at a concentration of 595 mg/m^3 for 30 min. The animals were killed at 80 min and 1, 3, 7, 10, 14, and 21 days after exposure. Rats retained Solvent Green 3 in their lungs for only 1 day. Rats killed at other times and mice and guinea pigs did not have Solvent Green 3 in their lungs at termination. The same species were exposed repeatedly to 500 mg/m^3, 1 hr/day for 5 days and killed 1 day and 2, 4, 6, and 8 weeks after exposure. Mice retained Solvent Green 3 in their lungs for only 1 day, rats retained the dye for 4 weeks, and the pulmonary macrophages in guinea pig retained the dye for 8 weeks (Marrs 1983).

Another group of animals were exposed to the dye mixture at concentrations of 105.8 mg/m^3 (low dose), 309.6 mg/m^3 (medium dose), and 1012.4 mg/m^3 (high dose for mice and rats) or 1162.1 mg/m^3 (high dose for guinea pigs), 5 days/week for 20 weeks (100 exposures) with sacrifices at 40 weeks (some mice) or 71 weeks after initiation of exposure (Marrs et al. 1984). In mice killed 40 weeks after initiation of exposure, Solvent Green 3 was retained primarily in the alveolar walls, and occasionally in alveolar spaces, in bronchial and bronchiolar lumina, and in arterial walls. The dye was also found in pulmonary macrophages. Solvent Green 3 was retained only in pulmonary macrophages in mice killed at 71 weeks; the number of animals with dye in their macrophages was significantly dose-related (P < 0.001) with 1/67 low-dose, 6/60 medium-dose, and 24/64 high-dose animals retaining the dye (Marrs et al. 1984).

The number of rats killed at 71 weeks that retained dye in pulmonary macrophages was also significantly dose related (P < 0.001). The results were as follows: 1/45 low-dose, 2/44 medium-dose, and 28/45 high-dose animals retained the dye. In guinea pigs killed at 71 weeks, the lungs of only 2 of 47 medium-dose animals had macrophages that contained dye (Marrs et al. 1984).

In all the animals examined by Marrs et al. (1984), Solvent Green 3 was found only in the respiratory tract. There was no evidence that the dye was absorbed into the blood, distributed to other tissues, or excreted in the urine or feces. Other reports discussed below confirm the observation that Solvent Green 3 is not absorbed but is retained in the lungs.

A detailed pharmacokinetics study was reported by Henderson et al. (1985a) and Medinsky et al. (1986). Fisher 344 rats were exposed for 60 min to aerosols of Solvent Yellow 33/Solvent Green 3 mixture by inhalation.

Solvent Green 3 was mixed and precipitated with Solvent Yellow 33, and the aerosols were generated by a modified Trost-Jet mill connected to the exposure chamber. The final concentration of dye in the exposure chamber was 246 ± 16 mg/m^3 (mean $\pm$ S.E.); the particle size expressed as mean median aerodynamic diameter (MMAD) was 2.6 μm with a geometric standard deviation of 1.7. The concentration of Solvent Yellow 33 in the aerosol mixture was 93 mg/m^3, and by substraction, the concentration of Solvent Green 3 was 154 mg/m^3. Animals were exposed to the aerosols and killed at various times up to 72 hr to determine the quantity of Solvent Green 3 retained in the lungs. The lungs were excised and homogenized in water. The homogenates were extracted with acetonitrile, and Solvent Green 3 was quantitated by HPLC.

A total of 360 μg (860 nmole) of Solvent Green 3 was deposited in the whole respiratory tract, 310 μg (740 nmole) in the upper respiratory tract and 50 μg (115 nmoles) in the lungs and bronchi. Solvent Green 3 was not cleared from the lungs during the sampling period, indicating that approximately 100 percent of the dye was retained. The estimated half-time of clearance was 22 days; the half-time could have been longer, but inadequate sampling times prevented an accurate calculation (Henderson et al. 1985a).

Solvent Green 3 was also retained in the lungs of rats exposed repeatedly to aerosols of Solvent Yellow 33/Solvent Green 3 mixture at concentrations of 11 ± 5 mg/m^3, 49 ± 11 mg/m^3, and 210 ± 50 mg/m^3 (mean $\pm$ S.D.) 6 hr/day, 5 days/week, for 4 weeks, and at 1.1 ± 0.5 mg/m^3, 10.2 ± 3.1 mg/m^3, and 101 ± 23 mg/m^3 (mean $\pm$ S.D.) 6 hr/day, 5 day/week, for 13 weeks (90 days) (Henderson et al. 1984b, 1985b). Three rats of each sex from each exposure group were killed 16 hr after the last exposure; the lungs were excised and homogenized. The homogenates were extracted with organic solvent, and Solvent Green 3 was analyzed by HPLC. The results are presented in Table 3. The percent retained represents the fraction of the total amount of Solvent Green 3 deposited in the lungs during the entire exposure period. A large fraction of the dye was retained, 14 to 33 percent after the 4-week exposure and 16 to 21 percent after the 13-week exposure.

To determine the rate at which Solvent Green 3 is cleared from the lungs, Henderson et al. (1985b) measured the amount of dye retained in the lungs of rats exposed to the high dose (101 mg/m^3) for 13 weeks and killed at various times after the last exposure. The results are presented in Table 4. A large fraction of the dye found in the lungs on the last day of exposure was retained for at least 230 days. The half-times of clearance calculated by fitting the data to a one-component exponential curve by least squares were 277 ± 67 days for males and 289 ± 40 days for females (Sun et al. 1987). These results demonstrate that Solvent Green 3 is cleared very slowly from the lungs.

4.1.2 Human Data

No data on the pharmacokinetics of Solvent Green 3 were found.

TABLE 3. RETENTION OF SOLVENT GREEN 3 IN LUNGS OF RATS EXPOSED REPEATEDLY BY INHALATION TO AEROSOLS OF SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE FOR 4 WEEKS[a]

Sex	Aerosol Conc. (mg/m^3)	Total Amount Retained[b]		Amount Deposited (mg/lung)	% Retained
		(μg/g lung)	(mg/lung)		
4 Weeks					
Male	0	1.0 ± 0.2	-	-	-
	11	290 ± 20	0.35 ± 0.04	1.0[c]	33
	49	740 ± 60	0.89 ± 0.07	4.7	19
	210	2110 ± 240	2.97 ± 0.3	20	15
Female	0	0.9 ± 0.3	-	-	-
	11	250 ± 30	0.24 ± 0.03	0.8	30
	49	650 ± 90	0.58 ± 0.09	3.5	16
	210	2150 ± 80	2.08 ± 0.18	15	14
13 Weeks					
Male	0	< 0.15	-	-	-
	1	43 ± 4	0.06 ± 0.003	0.32[d]	19
	10	480 ± 20	0.68 ± 0.3	3.2	21
	101	3400 ± 50	5.70 ± 0.08	31.8	18
Female	0	< 0.18	-	-	-
	1	39 ± 2	0.04 ± 0.001	0.24	17
	10	370 ± 40	0.41 ± 0.05	2.4	17
	101	2800 ± 200	3.86 ± 0.34	23.8	16

a. Adapted from Henderson et al. 1984b, 1985b.
b. Mean ± S.E.
c. Calculated by Henderson et al. (1984b) assuming 20 exposures, 6 hr/exposure, a minute volume of 200 mL for males and 150 mL for females, 10% lung and bronchial deposition, and 67% of Solvent Yellow 33/Solvent Green 3 mixture as Solvent Green 3.
d. Calculated by Henderson et al. (1985b) assuming 63 exposures, 6 hr/exposure, a minute volume of 200 mL for males and 150 mL for females, 10% lung deposition, and 70% of Solvent Yellow 33/Solvent Green 3 mixture as Solvent Green 3.

TABLE 4. LUNG RETENTION OF SOLVENT GREEN 3 IN RATS EXPOSED BY INHALATION TO 101 mg/m^3 OF SOLVENT GREEN 3 FOR 13 WEEKS[a]

Days After Exposure	Total Lung Content[b] (mg)	
	Male	Female
0	5.7 ± 0.1 (100)	3.9 ± 0.3 (100)
3	5.3 ± 0.6 (93)	3.6 ± 0.4 (94)
7	5.8 ± 0.8 (102)	3.9 ± 0.2 (100)
14	6.1 ± 0.5 (107)	3.6 ± 0.2 (92)
30	5.9 ± 0.2 (104)	3.3 ± 0.4 (85)
60	3.6 ± 0.4 (63)	3.4 ± 0.3 (87)
90	3.3 ± 0.5 (58)	2.8 ± 0.4 (72)
160	3.9 ± 0.5 (69)	2.6 ± 0.1 (66)
230	3.6 ± 0.5 (63)	2.1 ± 0.1 (55)

a. Adapted from Henderson et al. 1985b.
b. Mean ± S.E; numbers in parentheses are the percentages of 0-day values.

4.2 ACUTE TOXICITY

4.2.1 Animal Data

4.2.1.1 Oral, Dermal, and Ocular Toxicity

The acute oral LD$_{50}$ for Solvent Green 3 in rats was reported as >3.16 g/kg body weight in a toxicity study conducted by Hazelton Laboratories, Inc. (1962a). The dye was suspended in 0.5 percent methyl cellulose and administered by gastric intubation at doses of 10, 31.6, 100, 316, 1,000, or 3,160 mg/kg. The animals were observed for 7 days. The only effect reported was the presence of green dye in the feces. In another toxicity study, dogs were given a single capsule containing Solvent Green 3 at doses ranging from 10 to 1,000 mg/kg. Again, the only observed effect was green dye in the feces. The acute oral LD$_{50}$ was >1,000 mg/kg (Hazelton Laboratories, Inc. 1962b). Cichowicz and Wentzel (1983) reported oral LD$_{50}$'s of 10 and 15 g/kg in rats and 10 g/kg in rabbits and a minimal lethal dose of 6 g/kg in rabbits. They concluded that Solvent Green 3 has low acute oral toxicity in mammals. In a dermal toxicity study conducted by Hazelton Laboratories, Inc. (1965), Solvent Green 3 suspended in either petroleum or hydrophilic base at 0.1 or 1.0 percent was applied 15 times to abraded or intact skin of rabbits during a 3-week period. No local or systemic toxic effects were noted. All the other acute studies reviewed in this report involved exposure to Solvent Green 3 in a mixture with other dyes.

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An oral toxicity study was conducted in male and female Fisher 344 rats given 5 g/kg of Solvent Yellow 33/Solvent Green 3 mixture (24.1/70.9) in corn oil by gavage (Muni et al. 1986). The rats were observed 14 days for mortality and for signs of toxicity. All animals survived the 14-day observation period, and all animals showed a net weight gain, or only an insignificant weight loss. No gross internal lesions were observed. The fur of all animals was green on day 2, and the tails were also green on day 4. On day 14 the males were light green in color, and the females were yellow. Animals administered Solvent Yellow 33 alone also showed green and yellow external color changes. Therefore, the green external color can not be used as evidence of absorption or effects specifically related to exposure to Solvent Green 3. This study showed that the oral LD_{50} is greater than 5 g/kg.

In acute dermal toxicity tests, 2 g/kg of Solvent Yellow 33/Solvent Green 3 mixture was applied to shaved and abraded skin of five male and five female New Zealand rabbits for 24 hr (Muni et al. 1986). The animals were observed for 14 days. Body weights fluctuated by as much as 200 g, but were equal to or greater than pre-exposure weights on day 14. No significant skin or internal lesions were observed. One female, however, suffered from mild diarrhea (Muni et al. 1986).

In the primary dermal irritation test, 500 mg of Solvent Yellow 33/ Solvent Green 3 mixture was applied once to two abraded and two unabraded sites on six rabbits for 24 hr (Muni et al. 1986). Evaluation of the test site immediately after removal of the dye revealed only a barely perceptible erythema, which was resolved by 72 hr. The Primary Irritation score was 0.08, indicating that Solvent Yellow 33/Solvent Green 3 mixture was a very mild irritant.

The eye irritation test using 100 mg of Solvent Yellow 33/Solvent Green 3 powder placed in one eye of each of three rabbits showed that the dye was nonirritating to rabbit eyes (Muni et al. 1986).

4.2.1.2 Inhalation Toxicity

The toxicity of combustion products of nine different pyrotechnics, including a green smoke pyrotechnic unit containing Solvent Green 3 and auramine hydrochloride, was reported by Weeks and Yevich (1963). Ten male rats, ten female mice, and five each, male and female guinea pigs were exposed for 1 hr to green smoke produced by detonating pyrotechnic units inside static chambers. Relative low, medium, and high levels of concentration were established by the chamber size and the number of units fired. The particulate concentrations relative to exposure levels are shown in Table 5. At the medium- and high-exposure levels, the particulate concentration decreased very rapidly, with only 20 and 7 percent respectively, suspended in the chambers 15 min after detonation. Animals that survived exposure were observed for signs of toxicity and mortality for 7 days and sacrificed 4 weeks after exposure for histopathological evaluation (Weeks and Yevich 1963).

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TABLE 5. RELATIONSHIP OF EXPOSURE LEVEL TO PARTICULATE CONCENTRATION[a]

Exposure Level	Chamber Size (L)	No. of Charges	Particulate Concentration (mg/L)			
			3 min	15 min	30 min	45 min
Low	20,000	4	0.56	0.49	0.40	0.26
Medium	700	1	4.84	2.14	1.70	0.99
High	700	3	12.12	4.00	1.37	0.84

a. Adapted from Weeks and Yevich 1963.

The mortality rate of animals exposed to the highest concentration was as follows: 100 percent in rats, 70 percent in mice, 60 percent in female guinea pigs, and 0 percent in male guinea pigs. None of the animals exposed to the medium concentration died, but one rat exposed to the lowest concentration died.

In animals that survived, eye irritation, nasal discharge, gasping, and lethargy were observed. Weight gain was severely depressed in animals surviving the highest concentration, less severely depressed in animals exposed to the medium concentration, and only slightly depressed in animals exposed to the lowest concentration.

Gross and histopathological evaluations revealed heavy deposits of the dye in the nasal cavities and stringy masses of particulate matter adhering to the tracheal mucosa, which caused obliteration of bronchi and bronchioles in some animals. The pleural surfaces were green. The respiratory mucosa was necrotic and sloughing, and the alveolar spaces were edematous. The lungs were consolidated, and the surface of the lungs was mottled and green. Microscopic examination showed signs of chronic inflammatory proliferation, hypertrophy and metaplasia of the respiratory mucosa, in addition to the presence of alveolar macrophages. Gross lesions in the gastrointestinal tract appeared as ulcerated and hemorrhagic areas in the gastric mucosa and ulcerated areas in the duodenum. Microscopically, these lesions appeared necrotic or hemorrhagic.

Although toxic effects were observed in animals exposed to the green smoke, the effects could not be attributed to Solvent Green 3 alone, because the animals were also exposed to auramine hydrochloride.

Owens and Ward (1974) exposed seven animal species (monkey, dog, goat, swine, rabbit, rat, and guinea pig) to a single dose of a green smoke mixture composed of 70 percent Solvent Green 3, 20 percent benzanthrone, and 10 percent Vat Yellow 4. Six animals per exposure group (20 per group for rats and guinea pigs) animals were placed in a 20,000-L static chamber where grenades (between 1 and 12) were serially fired over a specified period of time in order to obtain a desired concentration x time (Ct). Concentrations

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ged from 3,346 to 13,085 mg/m^3, the exposure time ranged from 10 to 240
min, and the Ct's ranged from 78,159 to 1,330,090 mg·min/m^3. The animals
were observed 30 days for mortality and signs of toxicity. The LCt$_{50}$ (lethal
concentration x time) values were calculated from a Bliss analysis of the
mortality response data.

Toxic signs and time of onset after exposure to green smoke are pre-
sented in Table 6. All animals showed signs of respiratory injury followed
by generalized weakness, dyspnea, and prostration. The overall mortality
rates and LCt$_{50}$'s are presented in Table 7. During the first 24 hr, 47
percent of all animals died; all animals that died (52 percent) had done so
by day 16 after exposure. Owens and Ward (1974) attributed death to suffoca-
tion caused by overloading the airways with smoke particles. The LCt$_{50}$ for
all species combined (except goat) was 319,447 mg·min/m^3; for all nonrodents
(except goat), 314,856 mg·min/m^3; and for all rodents, 313,263 mg·min/m^3.
With goats eliminated from the calculations, the LCt$_{50}$'s are similar for all
groups. Based on LCt$_{50}$'s, the order of sensitivity is as follows: monkeys =
dogs > rats > guinea pigs > rabbits > swine > goats. Monkeys and dogs were
the most sensitive species, and goats were the most resistant.

TABLE 6. TOXICITY AND TIME OF ONSET IN SEVEN SPECIES FOLLOWING INHALATION
OF GREEN SMOKE DISSEMINATED FROM THE M18 GRENADE[a]

| Toxic Signs | Time of Onset (hr) | | | | | | |
	Dog	Swine	Goat	Monkey	Rabbit	Rat	G.P.[b]
Respiratory difficulty	0.5	1	1	1	0.5	0.5	0.5
Generalized weakness	0.5	1	1	1	0.5	0.5	0.5
Gagging	0.5	1	-	0.5	-	-	0.5
Vomiting	0.5	1	-	1	-	-	-
Dyspnea	1	1	1	1	1	1	0.5
Prostration	1	1	1	0.5	0.5	1	0.5
Death	1	1	1	0.5	0.5	0.5	0.5

a. Adapted from Owens and Ward 1974.
b. Guinea pig.

Henderson et al. (1985a) exposed specific pathogen free, male and
female Fischer 344 rats to aerosols of Solvent Yellow 33/Solvent Green 3
mixture (30:70 percent), generated by a Jet-O-Mizer air jet mill. The dye
was 95 percent pure; major contaminants included phthalic acid, phthalic
anhydride, quinaldine, quinazarin, and p-toluidine. Three animals of each
sex were exposed once for 1 hr at 1,600 mg/m^3 or once for 6 hr at 1,440
mg/m^3. Six animals of each sex were exposed repeatedly 6 hr/day for 5
days at 1,490 mg/m^3. Particle sizes, expressed as MMAD, ranged from 5.0
to 5.7 μm. Control animals were not included in this test. After ex-
posure, the animals were observed 14 days for mortality and signs of toxi-
city. All animals were weighed 7 and 14 days after completion of ex-
posure. The animals exposed repeatedly were subjected to gross necropsy,
and selected tissues were submitted for histopathological examination.

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TABLE 7. ACUTE TOXICITY IN SEVEN ANIMAL SPECIES FOLLOWING INHALATION OF
GREEN SMOKE DISPERSED FROM AN M18 GRENADE[a]

Species	Ct (mg·min/m^3)	Conc. (mg/m^3)	Exposure Time (min)	Mortality[b] (%)	LCt$_{50}$[c]
Monkey	83,661	3,346	25	0	
	201,476	4,797	42	33	
	224,254	4,771	47	17	224,678
	293,035	7,711	36	100	
	357,409	11,914	30	17	
	512,680	8,269	62	100	
Dog	78,159	4,342	18	0	
	201,478	4,797	42	33	
	224,254	4,771	47	33	230,637
	293,935	7,711	38	100	
	512,680	8,269	62	83	
	625,975	6,804	92	100	
Swine	201,478	4,797	42	0	
	224,254	4,771	47	17	
	403,972	6,516	62	0	
	457,973	13,085	35	17	452,968
	492,220	7,939	62	33	
	595,482	9,605	62	100	
	622,780	9,885	63	100	
Goat	201,478	4,797	42	0	
	224,254	4,771	47	0	
	492,220	7,939	62	0	919,917
	512,680	8,269	62	17	
	625,975	6,864	92	83	
	1,330,090	11,876	112	50	
Rabbit	210,478	6,568	32	0	
	332,780	11,885	28	33	
	403,972	6,516	62	100	
	409,117	10,885	38	100	340,245
	457,973	13,085	35	83	
	595,482	9,605	62	17	
Rat	83,661	3,346	25	0	
	210,160	6,568	32	5	
	332,780	11,885	28	65	297,955
	403,972	6,516	62	90	
	409,117	10,766	38	95	
	595,482	9,605	62	100	

TABLE 7. (cont.)

Species	Ct (mg·min/m³)	Conc. (mg/m³)	Exposure Time (min)	Mortality[b] (%)	LCt₅₀[c]
Guinea pig	83,661	3,346	25	0	
	210,160	6,568	32	5	
	332,780	11,885	29	15	324,431
	403,972	6,516	62	90	
	409,117	10,766	38	90	
	595,482	9,605	62	100	

a. Owens and Ward 1974.
b. Total number of animals: monkeys, dogs, goats, swine, and rabbits - 6/dose; rats and guinea pigs - 20/dose.
c. Lethal concentration x time causing 50% mortality; represents the Bliss statistical analysis of dose-response data.

All animals survived to the end of the test without overt signs of toxicity. One week after exposure, body weight decreased by 3 to 7 percent in all groups, but at the end of the observation period, body weights returned to pre-exposure levels.

Animals exposed repeatedly to aerosols of Solvent Yellow 33/Solvent Green 3 mixture suffered from nasal congestion. Compound-related microscopic lesions included slight to severe hyperplasia of the respiratory epithelium in the nasal cavity, serous inflammation of the naso-vomer organ with degenerative changes in the olfactory epithelium, and slight chronic nonsuppurative inflammation of the epithelium of the nasolacrimal duct. The dye also caused congestion in the lungs and focal alveolar histiocytosis in the lungs of almost all animals. Macrophages containing pigment were found in the tracheobronchial lymph nodes.

Henderson et al. (1985a) considered the lesion to be minor; consequently, they concluded that Solvent Yellow 33/Solvent Green 3 mixture has a low order of acute toxicity when inhaled by rats.

4.2.2 Human Data

No data were found on the acute toxicity of Solvent Green 3 in humans.

4.3.1 Animal Data

In a 6-week range-finding study in rats conducted by Hazelton Laboratories, Inc. (1962c), Solvent Green 3 was administered in the diet at concentrations of 0.1, 0.23, 0.55, 1.29, or 3.0 percent. The daily doses normalized against body weight were 119, 265, 638, 1460, or 3540 mg/kg at week 1 and 63.9, 165, 380, 902, or 2270 mg/kg, respectively, at week 6. There were no deaths or gross signs of toxicity. Food consumption and body weights were within normal range. The thyroid gland was small in animals fed the 0.55 and the 3.0 percent diets, but histopathological evaluation did not reveal abnormalities in the thyroid gland. Gross degenerative changes in the liver were observed and confirmed by histopathological examination, which showed an increase in vacuolated cells around the hepatic central vein. No other effects were observed.

In a 90-day subchronic study, two dogs were fed a diet containing 1 percent Solvent Green 3 during week 1 (290 mg/kg/day); the concentration was increased to 2 percent during week 3 (570 and 500 mg/kg/day) and 3 percent during week 5 (Hazelton Laboratories, Inc. 1962d). The weight-normalized doses fluctuated between 610 and 1400 mg/kg/day during the remainder of the experiment. There were no gross signs of toxicity, and no significant changes in body weight. Necropsy revealed only an accumulation of dye in the pelvis of the kidney, in the mucosa of the small and large intestines, in adipose tissue, and in the gall bladder. No histopathological alterations were observed; therefore, Solvent Green 3 was not found to be toxic under the conditions of this test.

Subchronic and chronic dermal toxicity tests revealed that 500 mg/kg of Solvent Green 3 in petroleum or hydrophilic base applied to rabbit skin for 13 weeks (Hazelton Laboratories, Inc. 1965), or single weekly applications of 1 mg of Solvent Green 3 in 0.1 mL of benzene to the skin of mice for 95 weeks did not cause significant local or systemic effects (Hazelton Laboratories, Inc. 1967). The thyroid glands in the male mice were enlarged, but histopathological examination did not show an effect.

Cichowicz and Wentzel (1983) described the results of a study in which rats and dogs were adminstered Solvent Green 3 in their diets at concentrations of 0.25, 1.0, or 3.0 percent for 2 years. They reported no effects even at the highest concentration. Other subchronic and chronic toxicity studies found in the literature and described below were conducted with mixtures comprised of Solvent Green 3 and other dyes.

In an inhalation toxicity study, Henderson et al. (1984b) exposed male and female Fischer 344 rats to aerosols of Solvent Yellow 33/Solvent Green 3 mixture (30:70) for 4 weeks. The mean measured aerosol concentrations were 11 ± 5 (low dose), 49 ± 11 (medium dose), and 210 ± 50 mg/m^3 (high dose), with particle sizes of 3.2 ± 0.4, 3.7 ± 0.5, and 4.9 ± 0.6 μm, respectively. The animals were observed for clinical signs of toxicity before initiation of exposure, 2 weeks after, and after

termination of exposure. Body weights and measurements of respiratory function were taken before and after termination of exposure; lung biochemistry, hematology tests, serum chemistry tests, and gross and histopathological evaluations were performed after termination of exposure (Henderson et al. 1984b).

No adverse gross clinical effects were observed. Male and female animals exposed to the high dose gained significantly less weight than controls. Male and female rats exposed to the medium and low doses, however, gained slightly more weight than controls.

Respiratory function tests were performed on 16 control and 16 high-dose animals. Absolute expiratory rates were significantly decreased, but the expiratory rates normalized against the forced vital capacity were not significantly altered. Other parameters significantly altered by exposure to the dye mixture were vital capacity normalized against total lung capacity (increased); residual volume, both absolute and normalized against total lung capacity (decreased); and diffusing capacity normalized against body weight or against alveolar volume (decreased). Henderson et al. (1984b) concluded that the dye mixture caused a decrease in lung volume, a reduction in gas exchange efficiency, and a slight airflow obstruction, but only in those animals exposed to the highest dose.

Evaluation of lung biochemistry by analysis of bronchoalveolar lavage (BAL) fluid showed that the following parameters were significantly elevated in high-dose rats: lactate dehydrogenase, ß-glucuronidase, alkaline phosphatase, glutathione reductase, glutathione peroxidase, acid proteinase, protein content, macrophages, and neutrophils (Table 8). Most of the acid proteinase activity was resistant to inhibition by leupeptin, indicating that the activity was cathepsin D. Protein content and neutrophils were elevated in medium-dose rats; macrophages and neutrophils were also elevated in low-dose rats.

Henderson et al. (1984b) suggested that the elevation in protein content and enzymes and the increase in macrophages and neutrophils in BAL fluid were indicative of an inflammatory response in high-dose animals. A mild inflammatory response in medium-dose animals was indicated by the increase in neutrophils. Henderson et al. (1984b) further suggested that the high level of cathepsin D, along with the more modest increase in cathepsin B, indicated that cleanup of lung particles and cellular debris was more important than turnover of pulmonary architecture.

Acid proteinase activity in lung tissue was elevated in animals exposed to the high dose of Solvent Yellow 33/Solvent Green 3 mixture. This activity was also   istant to leupeptin, indicating that it was cathepsin D; cathepsin B was not elevated in lung tissue. The neutral proteinases (plasminogen and cathepsin 6-polymorphonuclear leucocyte elastase) were moderately increased. According to Henderson et al. (1984b), these results were also indicative of an inflammatory response.

Hematology tests in 12 control rats and 12 rats exposed at each of the three dose levels revealed no changes. Serum chemistry tests showed that

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TABLE 8. ANALYSIS OF BRONCHOALVEOLAR LAVAGE (BAL) FLUID IN RATS EXPOSED TO SOLVENT YELLOW 33/
SOLVENT GREEN 3 MIXTURE BY INHALATION FOR 4 WEEKS[a]

Parameters[b]	Control	Exposure Concentration (mg/m^3)		
	Control	11	49	210
Lactate Dehydrogenase (mIU/g)	450 ± 40	460 ± 20	530 ± 50	1730 ± 80[c]
Acid Phosphatase (mIU/g)	19 ± 1	18 ± 1	16 ± 1	21 ± 1
Alkaline Phosphatase (mIU/g)	160 ± 12[d]	270 ± 15	260 ± 17	295 ± 15
β-Glucuronidase (mIU/g)	1.3 ± 0.2	1.2 ± 0.3	1.2 ± 0.2	12.1 ± 0.8[c]
Glutathione Reductase (mIU/g)	45 ± 6	50 ± 3	52 ± 3	99 ± 9[c]
Glutathione Peroxidase (mIU/g)	9.3 ± 1.6	9.3 ± 0.7	9.1 ± 0.8	15.0 ± 1.3[c]
Acid Proteinase (mg/hr/g)[e]	0.34 ± 0.05	0.25 ± 0.03	0.17 ± 0.03	1.85 ± 0.23[c]
Cathepsin D	0.13 ± 0.05	0.13 ± 0.02	0.16 ± 0.02	1.33 ± 0.14[e]
Cathepsin B	0.21 ± 0.10	0.13 ± 0.02	0.10 ± 0.04	0.55 ± 0.24
Protein (mg/mL)	1.4 ± 0.1	1.6 ± 0.1	2.0 ± 0.1[c]	3.6 ± 0.2[c]
Macrophages (10^3 cells/g)	450 ± 80	840 ± 100[e]	570 ± 60	1080 ± 150[c]
Neutrophils (10^3 cells/g)	2 ± 1	33 ± 6[e]	290 ± 94[c]	1720 ± 240[c]

a. Adapted from Henderson et al. 1984b.
b. Values are normalized to lung wet weight. Mean lung weights did not vary between groups.
c. $P < 0.05$, values differ from control value in multiple comparison t-test of the means.
d. Significantly different from the exposed groups in multiple comparison t-test, $P < 0.05$; current control value is low compared with historical controls.
e. Activity is reported as milligram of protein digested per hour per gram of lung.

serum alkaline phosphatase activity, total bilirubin, and creatinine were significantly elevated in all exposure groups, whereas inorganic phosphorus was elevated in animals exposed to the high dose. Cholesterol and glucose were elevated, but not significantly. The absence of histopathological changes in the liver, however, indicated that these changes in serum chemistry were not physiologically significant.

Histopathological evaluation of animals exposed to the highest dose showed a mild reaction around the terminal airways of the lungs that consisted of minimal to slight proliferation of foamy alveolar macrophages and minimal to slight hyperplasia of Type II pulmonary epithelial cells. This reaction was observed more often in males than in females, and was even observed in some medium-dose animals. Also in high-dose animals, clusters of reticuloendothelial cells with lymphoid hyperplasia were observed in the tracheobronchial lymph nodes, suggesting that, even in the absence of phagocytized particles, the dye had moved into the lymph nodes. A yellowish-brown pigment was found below the respiratory epithelium of the nasal septum and turbinates, but not in the larynx, trachea, or bronchi. No exposure-related lesions were observed in other organs (Henderson et al. 1984b). No exposure-related microscopic lesions were observed in rats exposed to Solvent Yellow 33 alone; therefore, the lesions observed in animals exposed to Solvent Yellow 33/Solvent Green 3 mixture may be caused by Solvent Green 3.

From these studies, Henderson et al. (1984b) concluded that the lowest-observed-effect level (LOEL) for aerosols of Solvent Yellow 33/Solvent Green 3 mixture was ≥ 50 mg/m^3; the no-observed-effect level (NOEL) was 11 mg/m^3.

Male and female Fisher 344 rats were also exposed to aerosols of Solvent Yellow 33/Solvent Green 3 mixture for 13 weeks (90 days) (Henderson et al. 1985b). Concentrations were 0 (control), 1.1 ± 0.5 (low-dose), 10.2 ± 3.1 (medium-dose), and 101 ± 23 mg/m^3 (high-dose), and particle sizes, expressed as MMAD, were 2.8 ± 0.4, 3.0 ± 0.2, and 4.2 ± 0.4 μm, respectively. Measurements and observations were made during exposure, immediately after exposure, and 30 days after termination of exposure.

Clinical observations 6 weeks after initiation of exposure, at termination of exposure, and after a 30-day recovery period showed no gross clinical effects or mortality. Body weights measured immediately after termination of exposure showed that high-dose males weighed 8.0 percent less than control males, and high-dose females weighed 9.2 percent less than control females. At the end of the 30-day recovery period, the body weight of high-dose male rats remained significantly lower than control males, whereas the body weight of high-dose female rats was similar to controls.

The parameters of respiratory function were the same as those measured in animals exposed for 4 weeks. There were no significant differences between values of absolute functions in control and exposed animals. Because body weights in high-dose animals were lower than control, there was a trend for variables normalized against body weight to be higher than

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those in control animals. Nevertheless, carbon monoxide diffusing
capacity normalized against body weight was the only variable signifi-
cantly altered (increased) (data were not shown). At the end of the 30-
day recovery period, the only variable significantly affected by exposure
was a lower carbon monoxide diffusing capacity normalized against alveolar
volume (0.016 mL/min/mm Hg/mL in high dose-animals, 0.020 in controls).
These results demonstrated that exposure to aerosols of Solvent Yellow
33/Solvent Green 3 mixture had very little effect on respiratory function
in rats.

Lung biochemistry was evaluated by analysis of BAL fluid 6 weeks after
initiation of exposure, immediately after termination of exposure, and
after the 30-day recovery period. Lactate dehydrogenase, ß-glucuronidase,
protein content, and the number of macrophages and neutrophils were
significantly affected by exposure to Solvent Yellow 33/Solvent Green 3
mixture (Table 9). In high-dose animals killed 6 weeks after initiation
of exposure, these effects did not become more severe but became less
severe with continued treatment and recovery. Except for the number of
macrophages, the values for the parameters were lower immediately after
termination of exposure than at 6 weeks after initiation of exposure.

TABLE 9. ANALYSIS OF BRONCHOALVEOLAR LAVAGE (BAL) FLUID IN RATS EXPOSED TO 101
mg/m^3 OF SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE BY INHALATION for 90 DAYS[a]

Parameter	Exposure	Sacrifice Week[b]		
		6	13	17
Lactate Dehydrogenase (mIU/g)[b]	Control	490 ± 40	390 ± 30	370 ± 20
	Exposed	1210 ± 50[c]	930 ± 50[c]	780 ± 40[c]
Acid Phosphatase (mIU/g)	Control	9.7 ± 0.8	9.2 ± 0.7	7.9 ± 0.6
	Exposed	16.1 ± 2.2	11.5 ± 1.0	7.4 ± 0.6
ß-Glucuronidase (mIU/g)	Control	1.5 ± 0.2	2.2 ± 0.8	0.9 ± 0.1
	Exposed	7.2 ± 0.3[c]	5.7 ± 0.8[c]	3.4 ± 0.5[c]
Protein (mg/mL)	Control	1.5 ± 0.2	1.1 ± 0.2	0.9 ± 0.1
	Exposed	3.4 ± 0 3[c]	3.2 ± 0.6[c]	2.0 ± 0 9[c]
Macrophages (10^3 cells/g)	Control	730 ± 60	600 ± 60	450 ± 40
	Exposed	770 ± 110	1000 ± 160[c]	580 ± 70
Neutrophils (10^3 cells/g)	Control	5 ± 2	0 ± 0	7 ± 3
	Exposed	1300 ± 130[c]	470 ± 100[c]	290 ± 50[c]

a. Adapted from Henderson et al. 1985b
b. Values represent total amount of material recovered in BAL divided by the
   net weight of the lung in grams; values are mean ± S.E., n = 12.
c. P ≤ 0.05, by Bonferroni pairwise comparison of means.

Acid proteinase activity in BAL fluid was not elevated. Acid proteinase activity in lung tissue, however, was significantly elevated in rats exposed to the high dose and killed immediately after termination of exposure. Although the level of activity decreased during recovery, it remained significantly higher than that in control animals. The changes observed in BAL fluid and lung tissues were indicative of an inflammatory reaction that did not clear up by the end of the recovery period. Henderson et al. (1985b) attributed the inflammation to Solvent Green 3 in the mixture and not to Solvent Yellow 33.

Serum chemistry and hematology tests revealed that alkaline phosphatase activity was significantly decreased, and cholesterol, glucose, inorganic phosphorus, total protein, and albumin were significantly increased in rats exposed to the high dose. Glucose, inorganic phosphorus, total protein, and albumin were elevated in medium-dose animals, and glucose, total protein, and albumin were elevated in low-dose animals. Because blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (SGPT), and creatinine levels were normal, indicating no damage to the kidneys and liver. Henderson et al. (1985b) concluded that these changes were not clinically significant. All serum chemistry parameters returned to normal by the end of recovery, indicating that the changes were also reversible.

Histopathological evaluation of animals exposed to Solvent Yellow 33/Solvent Green 3 mixture showed that in almost all high-dose animals, pigment was deposited in the submucosa of the nasal epithelium, the cortical tubules in the kidneys, and the bile duct epithelium or in hepatocytes adjacent to the bile duct. Lung lesions consisted of slight to moderate accumulation of foamy alveolar macrophages (containing pigment), accompanied by slight to moderate hyperplasia of Type II cells. Reticuloendothelial and lymphoid cells in the tracheobronchial lymph nodes were moderately hyperplastic.

In medium-dose animals, pigment was observed in the submucosal nasal epithelium. Minimal lesions in the lungs (alveolar macrophages and Type II cell hyperplasia) were observed. Reticuloendothelial and lymphoid cell hyperplasia with pigment deposition were also observed. No exposure-related lesions was observed in low-dose animals.

After the 30-day recovery period, lesions in the lungs in high-dose animals were slightly less severe than those observed immediately after exposure. In the nasal cavity and cortical tubules in the kidney pigment deposition was less severe, but in the liver it was unchanged. Reticulo-endothelial cell hyperplasia was more severe and lymphoid hyperplasia was unchanged.

In medium-dose animals, minimal lesions in the lungs were observed. Pigment deposition was observed in the nasal cavity, it was comparable to that of the controls in the kidney, but it was absent in the liver. Lymphoid hyperplasia was observed in one animal, but reticuloendothelial cell hyperplasia was absent in all animals. No exposure-related lesions were observed in the low-dose animals (Henderson et al. 1985b). In this

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study, the findings related to pigment deposition were similar to those observed after exposure to Solvent Yellow 33 alone; therefore, these effects could not be attributed to Solvent Green 3 in the mixture.

Because aerosols of Solvent Yellow 33/Solvent Green 3 mixture caused microscopic lesions in rats at the medium concentration (10 mg/m^3) and no exposure-related lesions at the low concentration, Henderson et al. (1985b) concluded that the NOAEL was 1 mg/m^3.

Marrs et al. (1984) described the toxic effects of chronic inhalation exposure to a smoke mixture composed of 19 percent Solvent Green 3, 13 percent Solvent Yellow 33, and 16 percent Disperse Red 9. Three animal species (400 Porton-strain SPF female mice, 200 Porton Wistar-derived female rats, and 200 Dunkin-Hartley female guinea pigs) were exposed to the combusted smoke mixture for 1 hr/day, 5 days/week for 20 weeks (100 exposures) at concentrations of 105.8 mg/m^3 (low dose), 309.6 mg/m^3 (medium dose), or 1,012.4 mg/m^3 (high dose, mice, rats) or 1,161.1 mg/m^3 (high dose, guinea pigs). Starting with the first exposure, the animals were observed for 71 weeks for toxic effects and then sacrificed for gross and histopathological evaluation. Because the animals were exposed to a mixture of dyes, toxic effects could not be attributed to Solvent Green 3 alone.

Treatment was discontinued in guinea pigs after 16 exposures, because of a high intercurrent mortality during exposure. The mortality rate at 71 weeks was 28 percent in exposed guinea pigs compared with 12 percent in the control group. Nevertheless, dose-related trends in mortality were not significant in high-dose guinea pigs, in other exposure groups, or in other species. Guinea pigs dying during exposure suffered from severe pulmonary congestion and alveolitis.

Body weights at the end of the observation period were significantly different only in rats exposed to medium and high doses. During the treatment period, mean body weights of exposed and control groups, as related to chronological age, were significantly different ($P < 0.005$, Kolmogorov-Smirnov test). Although the high-dose guinea pigs lost weight rapidly during the exposure, body weights stabilized after exposure was terminated.

Except for lungs, organ weights were not affected by treatment. The lungs in mice exposed to the high dose weighed more than lungs in mice exposed to medium and low doses ($P < 0.05$). The lungs in rats exposed to the high dose weighed more than those in control rats ($P < 0.001$).

Histopathological evaluation showed changes related almost exclusively to the respiratory tract in all animals dying prior to or surviving until termination. Dose-related lesions in mice sacrificed at 40 weeks included severe chronic pneumonia ($P < 0.05$), bronchiectasis ($P < 0.001$), and alveolitis ($P < 0.05$). These were nonspecific lesions, caused by inhaling particulate matter and not by specific toxic effects of the smoke mixture. Dose-related lesions found in mice at 71 weeks included the

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presence of alveolar macrophages (P < 0.001), mild to severe chronic pneumonia (P < 0.05), and fatty livers (P < 0.05).

Significant dose-related lesions in rats killed at 71 weeks included submucosal lymphocytes in the larynx (P < 0.05) and trachea (P < 0.01), perivascular lymphocyte aggregates (P < 0.0001), alveolitis (P < 0.05), and mild and severe foreign-body reaction characterized by the presence of alveoli packed with macrophages (P < 0.001). The foreign-body reaction often caused complete obliteration of alveolar spaces that should have caused a significant loss of respiratory capacity, and consequently, an increase in the mortality rate. The mortality rate, however, was not affected.

In guinea pigs, the incidence of severe alveolitis was increased in the low- and medium-dose groups (P < 0.05), but not in the high-dose group, which received only 10 exposures.

A discussion on hyperplastic and neoplastic lesions in animals exposed to this mixture is included in Section 4.6.

4.3.2 Human Data

No data were found on the subchronic or chronic toxic effects of Solvent Green 3 in humans.

4.4 GENOTOXICITY

4.4.1 Animal Data

In two surveys, one of anthraquinone derivatives and the other of cosmetic dyes, Solvent Green 3 was found to be nonmutagenic. Brown and Brown (1976) tested Solvent Green 3 at concentrations of 100 to 200 μg in 10 to 20 μL of dimethylsulfoxide (DMSO) per disc in *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, TA1978, and TA98, using the spot test. Muzall and Cook (1979) tested the dye (concentration not given) in strains TA1535, TA100, TA1537, and TA98 also using the spot test.

Moore et al. (1984) tested Solvent Yellow 33/Solvent Green 3 mixture (1:2 ratio, 95.0 percent pure) in seven strains of *Salmonella typhimurium*, in mouse lymphoma cells, and in mouse bone marrow cells. Solvent Green 3 was not tested alone. The in vitro tests were performed with and without activation with the S9 fraction from Aroclor 1254-induced rat liver.

Strains TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98 were tested in the Salmonella Reversion Assay, using the standard plate incorporation method. The dye was dissolved in DMSO and tested at the following concentrations: 0, 1, 5, 10, 30, 50, 100, 300, 500, and 1,000 μg/plate. Solvent Yellow 33/Solvent Green 3 mixture precipitated at 100

μg/plate; thus, the dose-response range was narrower and variations in the data were greater (Moore et al. 1984).

Strain TA100 gave a weak positive response with S9 activation and a negative response without S9 activation. Strain TA104 gave a weak positive response with and without S9 activation. Strain TA102 gave a strong positive response with and without S9 activation, and strains TA1535, TA1537, TA1538, and TA98 gave negative responses with and without S9 activation. Therefore, the data showed that Solvent Yellow 33/Solvent Green 3 mixture was mutagenic in three strains of Salmonella typhimurium, TA100, TA104, and TA102 (Moore et al. 1984). The results were very inconsistent; therefore, it was not possible to attribute the mutagenic response to either dye in the mixture.

The Mouse Lymphoma Assay, which detects mutations in the thymidine kinase locus, was conducted with L5178Y/TK$^{+/-}$ cell line at concentrations ranging from 2 to 40 μg/mL of Solvent Yellow 33/Solvent Green 3 mixture. Because of the limited solubility of the dyes in 1 percent DMSO, concentrations above 20 μg/mL were prepared in 2-percent DMSO. A positive response was indicated by a twofold increase in the mutant frequency at one or more concentrations from two separate assays and a dose-response relationship when cell survival was greater than 10 percent.

The results are summarized in Table 10. The dye mixture precipitated out of solution at concentrations of 6 μg/mL or more. Cell survival was

TABLE 10. SUMMARY OF MOUSE LYMPHOMA CELL MUTAGENICITY TESTS
WITH SOLVENT YELLOW 33/SOLVENT GREEN 3 MIXTURE[a]

| | Mutant frequency (x 10^6)[b] | | | | |
| | With S9 | | | Without S9 | |
Concentration	Test 1	Test 2	Test 3	Test 1	Test 2
Pos. Cont.[c]	374.7	252.2	384.8	531.9	898.7
1% DMSO	56.2	71.3	49.8	56.6	52.1
2% DMSO	58.4	68.9	46.0	46.6	NT
2 μg/mL	76.4	61.4	NT[d]	77.9	62.7
6 μg/mL	42.4	57.4	55.8	295.5	347.0
12 μg/mL	50.5	65.9	57.7	443.1	314.0
16 μg/mL	69.6	65.0	71.9	408.4	451.5
20 μg/mL	61.8	59.4	86.5	373.6	571.1
40 μg/mL[e]	150.7	140.3	274.4	381.6	NT

a. Adapted from Moore et al. 1984.
b. Total number of mutant colonies/number of viable cells plated.
c. Pos. Cont. = positive control, 40 μg/mL of 2-acetylaminofluorene (with S9); 15 μg/mL of methylmethanesulfonate (without S9).
d. NT = Not tested.
e. Because of low solubility, this concentration was prepared in 2-percent DMSO; all others were prepared in 1-percent DMSO.

greater than 10 percent at all concentrations except at 40 μg/mL in Test 1 where survival was only 9.2 percent. With S9 activation, Solvent Yellow 33/Solvent Green 3 mixture was mutagenic only at the highest concentration. Without S9 activation, the dye mixture was mutagenic at concentrations as low as 6 μg/mL.

Solvent Yellow 33 comprises one-third of the Solvent Yellow 33/Solvent Green 3 mixture. Other tests conducted by Moore et al. (1984) showed that the quantity of Solvent Yellow 33 in the dye mixture when tested without S9 (2 μg/mL) and with S9 (12 μg/mL) was also sufficient to elicit the mutagenic response when tested alone. Therefore, it is possible that Solvent Yellow 33 is the mutagenic component in Solvent Yellow 33/Solvent Green 3 mixture (Moore et al. 1984).

Moore et al. (1984) also analyzed the size distribution of the mutant colonies to determine whether the dye mixture was a mutagen, clastogen, or both. Small colonies represent chromosome damage (clastogenic effects), and large colonies represent single gene damage (mutations). The data showed that a large fraction of the mutant colonies induced by 20 μg/mL of Solvent Yellow 33/Solvent Green 3 mixture were small, indicating that the dye mixture induced chromosome damage. An analysis of the gross aberration frequency showed that 12 to 40 μg/mL of Solvent Yellow 33/Solvent Green 3 mixture induced 100 aberrations/100 cells. The types of aberrations consisted of chromosome breaks, translocations, and chromosome deletions. It should be noted that Solvent Yellow 33 alone also exhibited clastogenic effects.

In vivo sister chromatid exchange in male C57BL/6 mouse bone marrow cells was analyzed as another test for genotoxicity (Moore et al, 1984). Solvent Yellow 33/Solvent Green 3 mixture at doses of 10, 20, or 40 mg/kg in 0.1 mL DMSO + 0.1 mL corn oil was injected intraperitoneally (3 to 4 animals/group). Positive controls were injected with cyclophosphamide, and negative controls were injected with the vehicle. The results showed that none of the doses was effective in inducing sister chromatid exchange in bone marrow cells. The dye was not cytotoxic; nevertheless, Moore et al. (1984) presumed that the dye was distributed to bone marrow cells, although they found no traces of the dye in bone marrow cells. Solvent Yellow 33 alone was also ineffective in inducing sister chromatid exchanges in mouse bone marrow cells and in mouse lymphoma cells in vitro.

4.4.2 Human Data

No data were found on genotoxic effects of Solvent Green 3 in humans.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

4.5.1 Animal Data

Solvent Green 3 (D & C Green No. 6) was tested for teratogenic effects in rats and rabbits (Burnett et al. 1974). Doses based on the highest no-effect-level from previous long-term feeding studies in dogs and rats were administered during the critical stage of organogenesis. No effects on either the skeleton or soft tissues were observed. A multi-generation reproduction study was conducted in rats administered the dye in their diets at concentrations based on multiples of 1x, 10x, 30x, and 100x the ADI (acceptable daily intake) or on previous long-term feeding studies in dogs and rats; the doses, however, did not exceed 1,000 mg/kg (Pierce et al. 1974). No adverse effects on reproduction were observed.

4.5.2 Human Data

No data were found on the developmental or reproductive toxicity of Solvent Green 3 in humans.

4.6 ONCOGENICITY

4.6.1 Animal Data

No data were found on the carcinogenicity of Solvent Green 3 administered alone. Two studies, one on the carcinogenicity of Solvent Yellow 33 and Solvent Yellow 33/Solvent Green 3 mixture in mice, and another reporting the incidence of neoplastic lesions in animals exposed to a dye mixture containing Solvent Yellow 33, Solvent Green 3, and Disperse Red 9 were found.

Stoner (1985) tested Solvent Yellow 33/Solvent Green 3 mixture (24:71) in a lung tumor bioassay using strain A mice. A maximum tolerated dose (MTD) was established from the results obtained after injecting mice intraperitoneally with a dose of 25 mg/kg, six times over a 2-week period. Because this dose did not result in mortality or weight loss, the MTD was established at 25 mg/kg.

Solvent Yellow 33/Solvent Green 3 mixture was dissolved in trica-prylin and injected (intraperitoneally) at doses of 5, 12.5, or 25 mg/kg into 50 mice (25 males and 25 females) three times per week for 8 weeks. Untreated, vehicle-treated, and urethan-treated controls were included. The animals were killed 30 weeks after initiation of treatment and examined histologically for lung adenomas and tumors at other sites if gross lesions were observed.

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During the course of the study, 26 percent of the mice treated with Solvent Yellow 33/Solvent Green 3 mixture at a dose of 25 mg/kg died. Death was attributed to peritonitis caused by accumulation of dye in the peritoneal cavity. The results showed that the incidence of lung tumors was not increased, and tumors at other sites were not induced. Therefore, Solvent Yellow 33/Solvent Green 3 mixture was not carcinogenic in the lung tumor bioassay. Solvent Yellow 33 alone was also noncarcinogenic in this bioassay.

Marrs et al. (1984) exposed mice, rats, and guinea pigs to a smoke mixture containing 19 percent Solvent Green 3, 16 percent Disperse Red 9, and 13 percent Solvent Yellow 33. The animals were exposed for 1 hr/day, 5 days/week for 20 weeks at concentrations of 105.8, 309.6, or 1,012.4 mg/m^3 (rats and mice) or 1,161.1 mg/m^3 (guinea pigs). More details of the experimental procedure were presented in Section 4.3.

Seventy-one weeks after initiating treatment, histopathological evaluation revealed three lesions in medium-dose and two lesions in high-dose mice classified as hepatoma A, and one lesion in low-dose mice classified as hepatoma B; although no hepatomas were observed in control mice, a significant dose-related effect was not observed. One case of adenocarcinoma of the breast was observed in the low- and medium-dose groups, but none in controls; the incidence, however, did not indicate a significant dose-related trend.

In rats killed 71 weeks after initiating exposure, one adenocarcinoma and one squamous cell carcinoma of the lungs were observed, but a significant dose-related trend was not observed. In addition, two hemangiomas in the adrenal gland in the high-dose group (P < 0.05), one biliary hyperplastic lesion in the medium-dose and four in the high-dose groups (P <0.01), and three adenocarcinomas of the breast in the high-dose group were significant for dose-related trends. None of these neoplastic lesions were observed in controls. The incidence of neoplastic lesions in exposed guinea pigs was not significantly different from that of controls.

4.6.2 Human Data

No data were found on the oncogenicity of Solvent Green 3 in humans.

4.7 SUMMARY

No specific data were found on the pharmacokinetics of Solvent Green 3 administered orally. External color changes were observed in rats given Solvent Yellow 33/Solvent Green 3 mixture by gavage. Because the same external color changes are produced when rats are given Solvent Yellow 33 alone, this effect can not be attributed to Solvent Green 3, and thus, is not indicative of gastrointestinal absorption of the dye. The pharmacokinetics of Solvent Green 3 administered by inhalation show that the dye is retained in the respiratory tract for a very long time. The half-time

of clearance after a 90-day exposure is 277 days in male rats and 288 days in female rats.

No data were found on the pharmacokinetics of Solvent Green 3 in humans. Solvent Green 3 has a low order of toxicity whether administered acutely or chronically by the oral, dermal, or inhalation route. The acute oral LD_{50} is >3.16 g/kg in rats but may be as high as 15 g/kg, >1 g/kg in dogs, and 10 g/kg in the rabbit.

Acute inhalation exposure to very high concentrations (3,346 to 13,085 mg/m^3 for 18 to 112 min) of dye mixtures containing Solvent Green 3 may cause severe effects, such as respiratory difficulty, gagging, vomiting, weakness, prostration, and death. The acute LCt_{50} for monkeys, dogs, swine, rabbits, rats, and guinea pigs combined is 319,447 mg·min/m^3. Less severe effects restricted to the respiratory tract are caused by acute, subchronic, and chronic exposures at lower concentrations. Effects observed after a 4- and 13- week exposure to Solvent Yellow 33/Solvent Green 3 mixture and 20-week exposure to a mixture of Solvent Green 3, Solvent Yellow 33, and Disperse Red 9 are indicative of an inflammatory response in the respiratory tract.

Solvent Green 3 is not irritating to the eyes and is a very mild irritant to the skin.

Solvent Green 3 tested alone is not mutagenic in the Salmonella Reversion Assay. Solvent Yellow 33/Solvent Green 3 mixture may be mutagenic in **Salmonella typhimurium** in the presence or absence of S9 activation. In the Mouse Lymphoma Assay, Solvent Yellow 33/Solvent Green 3 mixture is mutagenic and clastogenic in both the presence and absence of S9 activation; the dye mixture, however, is more active in the absence of S9, suggesting that metabolism inactivates the mutagenic component. Because Solvent Yellow 33 alone is a potent mutagen, and the quantity of Solvent Yellow 33 in the mixture is sufficient to cause the observed positive response, the mutagenic component in the dye mixture may be Solvent Yellow 33. Solvent Yellow 33/Solvent Green 3 mixture is not active in the sister chromatid exchange assay in mouse bone marrow cells in vivo. There is no direct evidence to indicate that Solvent Green 3 is a mutagen.

Solvent Green 3 is not teratogenic in rats and rabbits. The dye is also not carcinogenic in the mouse lung tumor bioassay.

No data were found on the mutagenicity, teratogenicity, or carcinogenicity of Solvent Green 3 in humans.

Overall, the data indicate that Solvent Green 3 is a compound that has very little toxic effects in mammals.

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5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

Solvent Green 3, as D & C Green No. 6, is approved by the USFDA for use in medical devices and externally applied drugs and cosmetics. For use in coloring surgical sutures, the dye must conform to the following specifications: (1) not >2 percent volatile matter (at 135°C), (2) not >0.3 percent water-soluble matter, (3) not >1.5 percent matter insoluble in carbon tetrachloride, (4) not >0.5 percent intermediates, (5) not >10 ppm lead (as Pb), (6) not >1 ppm arsenic (as As), and (7) not <96.0 percent pure color. For coloring polyethylene terephthalate sutures used in general and ophthalmic surgery, D & C Green No. 6 should not exceed 0.75 percent by weight of the suture material. For use in polyglycolic acid sutures, the dye cannot exceed 0.1 percent by weight of the suture material (USFDA 1984).

For use in coloring externally applied drugs and cosmetics and contact lenses, D & C Green No. 6 must conform to the following specifications: (1), (2), and (3) as above; (4) not >0.1 percent p-toluidine, (5) not >0.2 percent 1,4-dihydroxyanthraquinone; (6) not >5.0 percent 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthracenedione; (7) not >20 ppm lead (as Pb); (8) not >3 ppm arsenic (as As); 9) not >1 ppm mercury (as Hg), and 10) not <96.0 percent total color. Levels of the dye used in contact lenses should not exceed 0.03 percent by weight of the contact lens material (USFDA 1984).

During the production of colored smoke grenades, workers are exposed to fine-powdered dusts. The U.S. Occupational Safety and Health Administration (USOSHA) standards (8-hr time-weighted average) for the levels of inert or nuisance dust in the occupational environment is 15 mg/m^3 of total dust or 5 mg/m^3 of respirable dust (USOSHA 1985). The American Conference of Governmental Industrial Hygienist (ACGIH 1986) and the International Labor Office (ILO 1980) recommended threshold limit values for inert or nuisance dust as 10 mg/m^3 of total dust or 5 mg/m^3 of respirable dust. The Federal Ambient Air Quality Standard for particulate matter is 75 μg/m^3 annual geometric mean and 260 μg/m^3 for a maximum 24-hr concentration not to be exceeded more than once per year (USEPA 1981, as reported in Cichowicz and Wentsel 1983).

The Surgeon General of the Army has established interim guidelines for the disposal of colored smokes. There should be no open burning, and personnel should not be exposed to dye components at levels above 0.2 mg/m^3 (8-hr time-weighted average) (Cichowicz and Wentsel 1983).

5.2 OCCUPATIONAL EXPOSURE

Manufacturing personnel are exposed to fine-powdered dusts through inhalation, skin, and eye contact. During training and testing

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operations, army personnel are exposed to pyrolysis reaction products formed during combustion of colored smoke grenades, and to dye vapors as condensates in the smoke cloud formed upon dissemination (Tatyrek 1965). According to Garcia et al. (1982), the levels of dust in the colored smoke grenade production facility at Pine Bluff Arsenal exceeded the limits established by USOSHA.

5.3 PREVIOUSLY CALCULATED CRITERIA

No aquatic or human health criteria have been previously calculated for Solvent Green 3.

5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by the USEPA for the estimation of water quality criteria for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value (FAV), whereas the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

Available data are insufficient to establish the FAV, and conse-quently, the CMC for Solvent Green 3. Acute toxicity tests with seven of the required eight freshwater aquatic species were conducted with a Solvent Yellow 33/Solvent Green 3 mixture (30:70). These data, however are not acceptable for determining the toxicity of Solvent Green 3 in fish and aquatic invertebrates for the following reasons: (1) data for a mixture cannot be used to determine the toxicity of a single compound and (2) Solvent Green 3 is insoluble in water and was not detected in test solutions by HPLC analysis at a detection limit of 0.002 mg/L. An accept-able acute test with Solvent Green 3 alone using a solvent to disperse the dye obtained a 96-hr TL_{50} of >100 mg/L for _Pimephales promelas_ (Chilling-worth 1974). Additional tests using a series of concentrations of purified Solvent Green 3 are necessary to determine more accurately the toxicity of this dye. The stock solution should be prepared by dissolving the dye in an appropriate solvent and diluting this solution to the desired series of concentrations. ASTM (1980) recommends that the concentration of solvent should not exceed 0.5 mL/L (a solvent control should also be tested) and that surfactants should not be used.

Acute testing with the green alga _Selenastrum capricornutum_ showed that 10 mg/L of Solvent Green 3 initially reduced algal growth to 17.8 percent of the control within 7 days, but recovery was almost complete by 14 days (Chillingworth 1974). No toxic effects were observed at 1 mg/L. An EC_{50} was not calculated for these data. Algal toxicity tests with a Solvent Yellow 33/Solvent Green 3 mixture (30:70) showed significantly reduced growth at concentrations of 0.198 mg/L of Solvent Yellow 33 and <0.002 mg/L of Solvent Green 3 (Fisher et al. 1987); however, these data

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are not acceptable for determining the toxicity of Solvent Green 3 alone. Therefore, data are presently insufficient to determine the Final Plant Value.

Because information is currently unavailable concerning the chronic toxicity or bioaccumulation of Solvent Green 3 in aquatic organisms, Final Chronic and Final Residue Values cannot be determined. Consequently, a CCC cannot be established for Solvent Green 3.

5.5 HUMAN HEALTH CRITERION

Neoplastic lesions were not found in mice treated topically with 1 mg of Solvent Green 3 dissolved in 0.1 mL of benzene once weekly for 95 weeks (Hazelton Laboratories, Inc. 1967) nor in rats and dogs administered diets containing Solvent Green 3 at concentrations of 0.25, 1.0, and 3.0 percent for 2 years (Cichowicz and Wentzel 1983). Solvent Yellow 33/Solvent Green 3 mixture at doses of 5, 12.5, and 25 mg/kg (intraperitoneal, 3 times/week, 8 weeks) was not carcinogenic in a mouse lung tumor bioassay (Stoner 1985). Neoplastic lesions were observed in laboratory animals exposed to Solvent Green 3 in a mixture with Disperse Red 9 and Solvent Yellow 33 (Marrs et al 1984). Nevertheless, carcinogenicity could not be attributed to the Solvent Green 3 in the mixture. No data were found on the carcinogenicity of Solvent Green 3 in humans. Therefore, sufficient data were not available for deriving a criterion based on carcinogenicity (nonthreshold chronic toxicity data) according to USEPA guidelines (USEPA 1980). The USEPA guidelines for deriving a water quality criterion for the protection of human health are summarized in Appendix B.

Adequate threshold chronic toxicity data in humans or laboratory animals were also not available for calculating a criterion. In a two-year toxicity study described by Cichowicz and Wentzel (1983), the animals were administered Solvent Green 3 in their diets. This method of exposure leads to inconsistent weight normalized doses. Nevertheless, Solvent Green 3 was not toxic at diet concentrations as high as 3 percent. In addition, in the absence of adequate pharmacokinetics data, we cannot conclude whether the dye was not absorbed from the gastrointestinal tract, or whether the dye was truly without toxic effects. In other chronic toxicity studies, the laboratory animals inhaled Solvent Green 3 in mixtures along with other dyes. Therefore, the data cannot be used to calculate a criterion based on threshold chronic toxicity data.

5.6 RESEARCH RECOMMENDATIONS

To satisfy the requirements established by the USEPA for deriving water quality criteria, the following research studies are recommended to fill gaps in the existing data:

1. To obtain more complete information for calculating the FAV, acute toxicity tests, following ASTM methods (ASTM 1980), should be performed for the remaining 7 families of aquatic organisms, as

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described by Stephan et al. (1985) (See Appendix A). Results from the acute test with the fathead minnow should be acceptable for the second family in the class Osteichthyes.

2. Chronic flow-through tests using measured concentrations for an invertebrate species, a fish species, and a sensitive freshwater species should be performed in order to calculate a Final Chronic Value.

3. Acute flow-through tests should be conducted using measured concentrations and following ASTM (1980) procedures for the three aquatic species for which chronic tests are also performed. This data will be used to calculate acute-chronic ratios.

4. To calculate an EC_{50} that will be used to determine a Final Plant Value, additional tests should be conducted with Selenastrum capricornutum using measured concentrations (prepared as serial dilutions from a stock solution in which the dye has been dissolved in an appropriate solvent). The end point should be growth inhibition.

5. Because the log K_p indicates the potential for significant bioaccumulation of Solvent Green 3, a definitive steady-state or 28-day bioaccumulation study should be conducted. A maximum permissible tissue concentration should be determined by conducting a chronic wildlife feeding study or a long-term wildlife field study. These data will provide necessary information for the calculation of a Final Residue Value.

6. Solvent Green 3 exhibits negligible water solubility; consequently, the dye will probably exist in aquatic systems in a particulate form, which could remain suspended or settle out and be deposited in bottom sediments. Because burrowing organisms and bottom-feeders may be exposed to high concentrations of the dye, it is suggested that sediment bioassays be performed with Hexagenia (Insecta: Ephemeroptera) using the modified apparatus described in Fremling and March (1980, pp. 91-92). In addition, studies should be undertaken to determine the fate of the dye in aquatic sediments (i.e. sorption kinetics, partitioning between sediment and water phases, and potential pathways of degradation).

7. All mammalian toxicity tests described below should be conducted with Solvent Green 3 alone. A well-designed pharmacokinetics study should be performed in rats administered Solvent Green 3 by gavage. If the data show that Solvent Green 3 is not absorbed or is absorbed at an exceptionally low rate from the gastrointestinal tract, then additional oral chronic toxicity tests are not recommended, but a 2-year inhalation test should be performed.

8. If the pharmacokinetics data show that Solvent Green 3 is absorbed from the gastrointestinal tract, then 2-year chronic toxicity/carcinogenicity tests should be performed in rats. The dye should be administered by gavage, using doses based on the projected blood and tissue levels, and the tests should be performed according to USEPA Toxic Substances Control Act Test Guidelines (USEPA 1985).

9. If Solvent Green 3 is absorbed, the following additional tests should be performed: in vitro mutagenicity tests in mouse lymphoma cells, dominant lethal assays in rats and/or in vivo chromosome aberration assays in mouse bone marrow cells, and reproductive/developmental toxicity tests in rats.

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7. GLOSSARY

ACGIH   American Conference of Governmental Industrial Hygienists

ASTM   American Society for Testing and Materials

BAL   Bronchoalveolar lavage

BUN   Blood urea nitrogen

CCC   Criterion Continuous Concentration

CMC   Criterion Maximum Concentration

Ct   Concentration x time

DMSO   Dimethylsulfoxide

DO   Dissolved oxygen

EC_{50} Effective concentration causing 50 percent inhibition of algal growth

FAV   Final Acute Value

HPLC   High performance liquid chromatography

ILO   International Labor Office

LD_{50} Lethal dose causing 50 percent mortality

LCt_{50} Lethal concentration x time causing 50 percent mortality

LDH   Lactate dehydrogenase

LOEL   Lowest observed effect level

$Log\ K_p$ Octanol-water partition coefficient

MMAD   Mass median aerodynamic diameter

MTD   Maximum tolerated dose

NOEL   No observed effect level

SGPT   Serum glutamic pyruvic transaminase

GLOSSARY (Continued)

SY	Solvent Yellow 33
SY/SG	Solvent Yellow 33/Solvent Green 3 mixture
TL_{50}	Mean tolerance limit
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
USOSHA	United States Occupational Safety and Health Administration
USITC	United States International Trade Commission
USTC	United States Tariff Commission

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA
FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect aquatic life and is slanted towards the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedure and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species all of the time was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species was very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different non-ionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

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NOTE: Analytical chemistry of the material may have to be considered
when defining the material or when judging acceptability of some
toxicity tests, but a criterion should not be based on sensitivity of
an analytical method. When aquatic organisms are more sensitive than
analytical techniques, the proper solution is to develop better
analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity
   to, and bioaccumulation by, aquatic animals and plants: (b) FDA
   action levels (FDA Guidelines Manual): and (c) chronic feeding
   studies and long-term field studies with wildlife that regularly
   consume aquatic organisms.

2. All data used should be available in typed, dated and signed hardcopy
   with enough supporting information to indicate that acceptable test
   procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but
   data on formulated mixtures and emulsifiable concentrates of the test
   material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it
   may be appropriate to only use results of flow-through tests in which
   concentration of test material in test solutions were measured using
   acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have
   reproducing wild populations in North America, or organisms that were
   previously exposed to significant concentrations of the test material
   or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater
   animals in at least eight different families such that all of the
   following are included:

   a. the family Salmonidae in the class Osteichthyes:

   b. a second family (preferably an important warmwater species) in the
      class Osteichthyes (e.g., bluegill, fathead minnow, or channel
      catfish):

   c. a third family in the phylum Chordata (e.g, fish or amphibian):

   d. a planktonic crustacean (e.g, cladoceran or copepod):

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e. a benthic crustacean (e.g., ostracod, isopod, or amphipod):

f. an insect (e.g., mayfly, midge, stonefly):

g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca): and

h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L) should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

   a. Tests with daphnids and other cladocerans should be started with organisms < 24 hr old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

   b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96- hr LC_{50} should be used in place of the desired EC_{50}.

   c. Tests with single-cell organisms are not considered acute tests, even if the duration was ≤ 96 hr.

   d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate a that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison to other acute and chronic data for the species and other species in the same genus probably should not be used.

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9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentration of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as $R/(N+1)$.

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln\ GMAV)^2) - ((\Sigma(\ln\ GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln\ GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured **is lower than the FAV, then that geometric mean should be used as the FAV.**

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are

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actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^Y$.

11. Obtain the FAV at Z by using the procedure described in Section 5. (No. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as:

$$FAV = e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])},$$

where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

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4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic do's not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

   a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young < 48 hr old, continue through maturation and reproduction, and should end rot < 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young < 24 hr old and last for not < 21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

   b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

   c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

   NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (No. 10-14). Then go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used. If acute tests were not conducted as part of the same study, acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

   a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

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b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute--Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and salt-water species.

c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are < 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z by the Final Acute-Chronic Ratio.

c. Use V — pooled acute slope as L — pooled chronic slope.

d. Go to Section 8, No. 2, item m.

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7. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

  a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

  b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

  c. Decide whether data for each species is useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

  d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

  e. Similarly normalize the values of the water quality characteristic for each species individually.

  f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

  g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

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h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related valuₓ; of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M \cdot L(\ln P \cdot \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^Q$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (No. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as:

$$FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S \cdot L[\ln Z])},$$

where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation

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factors. A maximum permissible tissue concentration is either (a) a
FDA action level (FDA administrative guidelines) for fish oil or for
the edible portion of fish or shellfish or (b) a maximum acceptable
dietary intake (ADI) based on observations on survival, growth, or
reproduction in a chronic wildlife feeding study or a long-term
wildlife field study. If no maximum permissible tissue concentration
is available, go to Section 11., because a Final Residue Value cannot
be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs)
   are the quotients of the concentration of a material in one or more
   tissues of an aquatic organism divided by the average concentration
   in the solution to which the organism has been exposed. A BCF is
   intended to account only for net uptake directly from water, and thus
   almost has to be measured in a laboratory test. A BAF is intended to
   account for net uptake from both food and water in a real-world
   situation, and almost has to be measured in a field situation in
   which predators accumulate the material directly from water and by
   consuming prey. Because so few acceptable BAFs are available, only
   BCFs will be discussed further, but an acceptable BAF can be used in
   place of a BCF.

3. If a maximum permissible tissue concentration is available for a
   substance (e.g. parent material or parent material plus metabolite),
   the tissue concentration used in BCF calculations should be for the
   same substance. Otherwise the tissue concentration used in the BCF
   calculation should be that of the material and its metabolites which
   are structurally similar and are not much more soluble in water than
   the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF
      was calculated based on measured concentrations of test material
      in tissue and in the test solution, and exposure continued at
      least until either apparent steady-state (BCF does not change
      significantly over a period of time, such as two days or 16
      percent of exposure duration, whichever is longer) or 28 days was
      reached. The BCF used from a test should be the highest of (a)
      the apparent steady-state BCF, if apparent steady-state was
      reached; (b) highest BCF obtained, if apparent steady-state was
      not reached; and (c) projected steady-state BCF, if calculated.

   b. Whenever a BCF is determined for a lipophilic material, percentage
      of lipids should also be determined in the tissue(s) for which the
      BCF is calculated.

   c. A BCF obtained from an exposure that adversely effected the test
      organisms may be used only if it is similar to that obtained with
      unaffected individuals at lower concentrations that did cause
      effects.

   d. Because maximum permissible tissue concentrations are rarely based
      on dry weights, a BCF calculated using dry tissue weights must be

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converted to a wet tissue weight basis. If a conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

    c. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, then the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

    a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

    b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

    a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

    b. Calculate the geometric mean normalized BCF.

    c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

       • For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.

       • For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

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- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lower of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as: The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) $\mu g/L$ more than once every three years on the average and if the one-hour average concentration does not exceed (4) $\mu g/L$ more than once every three years on the average.

Where,

    (1) - insert freshwater or saltwater,

    (2) - name of material,

    (3) - insert the Criterion Continuous Concentration, and

    (4) - insert the Criterion Maximum Concentration.

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SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA
FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

  (a) Carcinogenicity, (b) Toxicity, and (c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish. Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to

be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980). Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCFavg - BCFsp \times \frac{3.0\%}{PLsp} ,$$

where BCFsp is the bioconcentration factor for an aquatic species and PLsp is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF - (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to 10^{-5} (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065\ BCF)}$$

where,

C = ambient water concentration;
PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
BCF = the bioconcentration factor; and
q_1^* = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C\ (2 + 0.0065\ BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065\ BCF \times C)}{70}$$

where,

2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 x BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* \ X \ ,$$

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where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X} ,$$

showing that the coefficient q_1^* is the ratio of risk to dose: an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/-response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2   CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk $[RR(X)]$ of a cohort of individuals at exposure X compared to the risk in the control group $[PR(control)]$ (e.g., if the cancer risk in group A is five times greater than that of the control group, then $RR(X) = 5$). In such cases the "excess" relative cancer risk is expressed as $RR(X) - 1$, and the actual numeric, or proportional excess risk level $[PR(X)]$ can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control).$$

Using the standard risk/dose equation:

$$PR(X) = b \times X$$

And substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X}$$

where b is equal to the carcinogenic potency or q_1^*.

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3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest index of cancer potency (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3} .$$

3. The dose must also be normalized to a lifetime average exposure. For an carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is le, and the total length of the experiment is Le, then the lifetime average exposure (Xm) is

$$Xm = \frac{l_e \times m}{L_e \times W^{2/3}}$$

4. If the duration of the experiment (Le) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of (L/Le)3 to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r ,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW ,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man: 0.05 for a 0.35-kg rat: and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

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6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol, and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption: which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t:d) = 1 - \exp(-g(d)H(t)) ,$$

where $P(t:d)$ is the probable response for dose d and time t: $g(d)$ is the polynomial function defining the effect of dose level, and $H(t)$ the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i$$

$$H(t) = \sum_{i=0}^{b} \beta_i t^i$$

(with α and $\beta \geq 0$, and $\Sigma \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each S as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp(- \sum_{i=0}^{a} \alpha d^i),$$

or as given in the EPA guidelines (USEPA 1980):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots q_k d^k)] ,$$

where $P(d)$ is the lifetime risk (probability) of cancer at dose d.

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For a given dose the excess cancer risk $A(d)$ above the background rate $P(o)$ is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

where,

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function $A(d)$ at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function $A(d)$ has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus $A(d)$ and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_o - L_1) = 2.70554,$$

where Lo is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where Ni is the number of animals in the ith dose group, Xi is the number of animals in the ith dose group with a tumor response, Pi is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (X_2) is larger than the cumulative 99 percent point of the chi-square

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distribution with f degrees of freedom where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at, and below which, the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065 \text{ kg} \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criteria.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

     (1) NOEL — No-Observed-Effect-Level,
     (2) LOEL — Lowest-Observed-Effect-Level,
     (3) LOAEL — Lowest-Observed-Adverse-Effect-Level,
     (4) FEL   — Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If a LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to a NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only a NOEL and a LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, NOAEL, LOAEL, and clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported,

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but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criteria (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times AA}{(AO \times SF)}$$

where,

BR — daily air intake (assume 10 m3),
DE — duration of exposure in hours per day,
d — 5 days/7 days,
AA — efficiency of absorption from air,
AO — efficiency of absorption from oral exposure, and
SF — safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{CA \times DE \times d \times AA \times BR \times 70 \text{ kg}}{(BWA \times AO \times SF)}$$

where,

CA	=	concentration in air (mg/m3),
DE	=	duration of exposure (hr/day),
d	=	number of days exposed/number of days observed,
AA	=	efficiency of absorption from air,
BR	=	volume of air breathed (m3/day),
70 kg	=	standard human body weight,
BWA	=	body weight of experimental animals (kg),
AO	=	efficiency of absorption from oral exposure, and
SF	=	safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant: however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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